Biodiversité et syndrome de dispersion dans les communautés de macrofaune du sol

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Habilitation à diriger des recherches

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Comprendre et prédire la distribution spatiale de la biodiversité et sa dynamique est un des objectifs centraux de l'écologie des communautés. Cette problématique est un enjeu actuel essentiel car les changements globaux représentent un véritable défi pour les écosystèmes et les espèces.

De nombreux travaux montrent qu'il existe une relation positive entre diversité (spécifique et fonctionnelle) et fonctionnement des écosystèmes. La question sous-jacente est donc d'identifier les déterminants de cette diversité. Comprendre ces déterminants dans le groupe de la macrofaune du sol (organismes de taille supérieure à 2mm à l'âge adulte) a été le fil conducteur des travaux que j'ai réalisés.

Cette problématique a été peu explorée chez la macrofaune du sol au niveau spécifique, alors que ce groupe constitue une partie essentielle de la biomasse et de la diversité des organismes terrestres. Ce manque de connaissances vient en partie des difficultés inhérentes à l'étude des organismes du sol. Par ailleurs la plupart des prédictions écologiques se voulant générales ont été formulées à partir d'observations sur des organismes de surface, en ignorant les organismes du sol alors que ceux-ci sont soumis à des contraintes extraordinairement plus complexes et éloignées de celles présentes en surface ou en milieu purement aquatique. La généralité de ces principes « généraux », développés sur les organismes dans des milieux de vie somme toute spécifiques, se doit d'être testée dans cet autre milieu, le compartiment du sol.

Ceci m'a amené à suivre deux axes de recherche :

Un premier axe de recherche m'a conduit à développer et coordonner des recherches sur les mécanismes clés de la distribution spatiale des espèces, déjà identifiés chez d'autres organismes mais encore peu connus chez les organismes du sol. Ces différents travaux ont été guidés par les travaux empiriques et théoriques développés sur les organismes aquatiques et de surface, et ont porté principalement sur la dispersion des organismes.

Un second axe d'étude a porté sur la recherche de mécanismes méconnus de distribution spatiale des organismes du sol, et de questionner la généralisation de ces mécanismes aux organismes aquatiques et de surface. Nous verrons ainsi que le mécanisme de modification de l'environnement de manière non trophique, l' "ingénierie écologique", me parait être un aspect central, mais souvent négligé, dans la formation des assemblages d'espèces de manière générale. Ces résultats sont le fruit de travaux collectifs et sont maintenant présentés.

Remerciements

Je tiens à remercier vivement les membres du jury qui ont accepté de donner un peu de leur précieux temps pour cette occasion.

Merci encore à Patrick Lavelle puis Luc Abbadie pour m'avoir accueilli dans leur laboratoire, devenus maintenant IEES, une structure riche, stimulante et très prometteuse.

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Merci à tous les étudiants qui ont contribué à faire les recherches présentées ici, en particulier aux doctorants, Gael Caro, Benoit Richard, et les M2 : Christophe Lecarpentier, Caroline Thiollet, Raphael Marichal, Gabrielle Ringot. Ils se sont donnés à fond pour obtenir les résultats synthétisés ici en quelques pages!

Enfin une pensée affectueuse pour ma petite famille qui vient de s'agrandir!

INTRODUCTION

RÔLE DE LA DISPERSION DANS LA FORMATION DES COMMUNAUTÉS : ÉMERGENCE DE MÉTACOMMUNAUTÉS

Deux concepts ont permis une avancée significative dans la compréhension de la distribution spatiale de la biodiversité. Il s'agit des concepts de communauté écologique, et de métacommunauté.

Une communauté écologique peut être définie comme un assemblage d'espèces qui interagissent et coexistent de manière transitoire dans l'espace et dans le temps. Une communauté peut être décrite par la composition et la diversité des espèces ainsi que par les caractéristiques (souvent appelés "traits") qui forment le phénotype des individus. Dans la pratique on considère souvent des communautés formées par des organismes de la même guilde, c'est-à-dire ayant grosso-modo le même type de régime alimentaire, mais en réalité le concept ne se limite pas à ce cas de figure : on peut considérer un assemblage de proies et prédateurs par exemple. Un aspect intriguant de la biodiversité est la régularité de la structure des communautés, avec la présence de nombreuses espèces rares et de quelques espèces (ultra) dominantes. Quels mécanismes aboutissent à cette régularité? Deux types de mécanismes ont été avancés pour expliquer ce phénomène:

- Les mécanismes internes à la communauté, c'est-à-dire des mécanismes ayant trait
  - à l'écologie des espèces (niche écologique : species sorting hypothesis)
  - aux interactions entre espèces, avec une certaine emphase sur la compétition intra et interspécifique (théorie de la compétition, principe d'exclusion compétitive, ressource ratio hypothesis, réseaux d'interactions).
  - aux relations trophiques présentes dans la communauté: approche réseaux trophiques.

- Les mécanismes opérant à une échelle plus large que la communauté, c'est-à-dire ceux qui font appel à l'aspect spatial des communautés. Dans cette optique les échanges d'individus entre communautés sont mis en avant, ce qui génère des dynamiques plus complexes et nécessite d'appréhender les communautés à un degré d'organisation supérieur, que l'on appelle métacommunautés.

Au final les communautés écologiques sont généralement considérées comme la résultante de l'action de trois types de déterminants : les interactions entre organismes, l'environnement, et les flux d'individus (la dispersion, *Figure 1*).

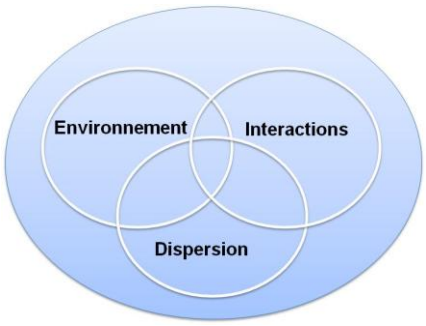


FIGURE 1 LES TROIS DÉTERMINANTS DE LA COMPOSITION DES COMMUNAUTÉS ÉCOLOGIQUES.

Déterminer l'influence relative de chaque type de déterminant est malaisé car il est difficile de les manipuler individuellement, et indépendamment les uns des autres. Par ailleurs, à certaines échelles spatiales toute expérimentation est impossible à mettre en œuvre. En conséquence l'influence de ces différents déterminants est souvent mise en évidence de manière indirecte, en confrontant les données observées aux prédictions de modèles théoriques faisant des hypothèses sur les mécanismes en jeu.

Chez la macrofaune du sol, l'influence de l'environnement a été beaucoup plus étudiée que le rôle des interactions ou de la dispersion (Eijsackers, 2011). Ceci résulte probablement du fait que la dispersion est très difficile à étudier, en particulier pour les organismes souterrains. Pour s'en convaincre on peut regarder le nombre d'articles publiés comportant le mot "dispersion", "compétition" ou "habitat" pour différents groupes d'organismes du sol. Pour cela on utilise le mot clé dispersal, compétition ou habitat et en excluant les mots "seed" et "plant" à cause du grand nombre d'articles relatant de la dispersion des graines et des plantes. Il apparaît (Tableau **1**) alors que chez tous les groupes de la macrofaune du sol, les études sont dominées par des travaux en relation avec l'habitat, ensuite la compétition, et souvent loin derrière la dispersion. Même si cette analyse est un peu grossière et non exhaustive, elle relève tout de même une tendance forte à négliger les processus de dispersion chez la macrofaune du sol.

Ces constatations m'ont amené à orienter mes recherches sur le rôle de la dispersion sur la biodiversité des macro organismes du sol. La dispersion pouvant jouer un rôle à différentes échelles spatiales, j'ai balayé ces différentes échelles au fil des différentes études.

Groupe	mots clés	nombre d'articles
fourmis	*ant dispersal*	129
	ant competition	952
	ant habitat	1439
	ant	33634
termites	*termite dispersal*	2
	termite competition	109
	termite habitat	341
	termite	7465
araignées	*spider dispersal*	134
	spider competition	702
	spider habitat	1441
	spider	23965
coleoptères	*coleoptera dispersal*	69
	coleoptera competition	899
	coleoptera habitat	2922
	coleoptera	43945
diplopodes	*millipede dispersal*	14
	millipede competition	25
	millipede habitat	99
	millipede	1261
chilopodes	*centipede dispersal*	13
	centipede competition	21
	centipede habitat	59
	centipede	666
vers de terre	*earthworm dispersal*	22
	earthworm competition	104
	earthworm habitat	388
	earthworm	9508
isopodes	*isopod dispersal*	7
	isopod competition	5
	isopod habitat	38
	isopoda	2845
hymenoptères	*hymenoptera dispersal*	16
	hymenoptera competition	25
	hymenoptera habitat	130
	hymenoptera	37830
Blattoptères	*Blattodea dispersal*	6
	Blattodea competition	6
	blatoodea habitat	25
	Blattodea	341
lézards	lizard dispersal	473
	lizard competition	649
	lizard habitat	2156
	lizard	19611
oiseaux	bird dispersal	3155
	bird competition	3750
	bird habitat	14871
	bird	210565

TABLEAU 1 **Nombres d'articles trouvés dans Web of Science relatant de la dispersion, de la compétition et de l'habitat chez la macrofaune du sol et deux groupes externes, trois forces potentiellement importantes pour la structuration des communautés.**

1. PATTERNS DE BIODIVERSITÉ ET DISPERSION DE LA MACROFAUNE DU SOL À DIFFÉRENTES ÉCHELLES

1.1 DISTRIBUTION SPATIALE DES COMMUNAUTÉS DE VERS DE TERRE À L'ÉCHELLE DE LA FRANCE

La description des variations spatiales de la biodiversité constitue une étape fondamentale dans la compréhension de la distribution des espèces et est une étape préliminaire nécessaire pour aller vers des modèles prédictifs. Étonnamment peu de données existent sur la distribution spatiale de la macrofaune du sol, en particulier chez les vers de terre, à échelle moyenne ou grande, avec une bonne résolution spatiale. En conséquence certains aspects fondamentaux de la distribution spatiale de la diversité de la macrofaune du sol restent encore largement inconnus. Dans le travail présenté dans ce chapitre (Mathieu & Jonathan Davies, 2014, Annexe 1), nous exploitons un jeu de données sur la distribution des vers de terre en France, publié par M. Bouché (Bouché, 1972). Cette base comporte l'abondance de toutes les espèces de vers de terre français sur plus de 1300 points distribués de manière homogène sur l'ensemble de la métropole. (Figure 2). Cette base de données est unique de par l'ampleur de l'échantillonnage qu'elle représente - qui a nécessité plusieurs années de travail - mais aussi et peut être surtout du fait de la fiabilité et de l'homogénéité de l'identification des spécimens entièrement réalisée par M.Bouché.

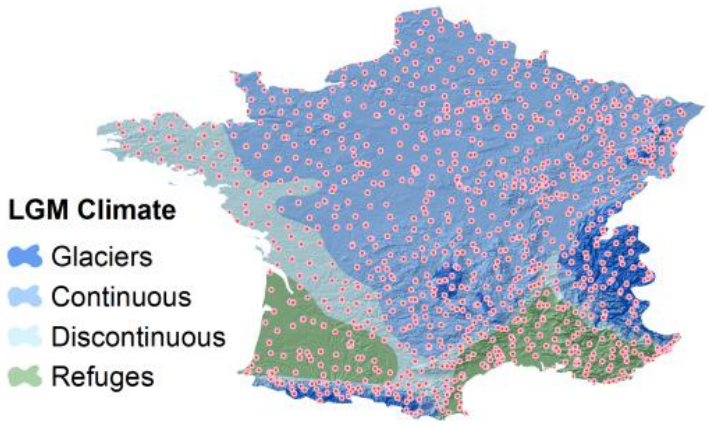


FIGURE 2 Carte des points d'échantillonnage des vers de terre en France, par M. Bouché (Bouché, 1972). Les conditions climatiques du sol pendant les dernières glaciations (LGM, ~ -16000 ans) sont indiquées: glaciers : zones en permanence recouvertes d'un glacier, Continuous : sols gelés en permanence (permafrost), Discontinuous : zones d'alternance gel – dégel du sol, Refuges : sols jamais gelés.

Nous avons exploité cette base afin d'explorer chez les vers de terre trois questions centrales en biogéographie, et discutons par la suite des mécanismes qui ont pu aboutir à cette distribution, avec une emphase sur le rôle de la dispersion.

Q°1 Retrouve-t-on un gradient latitudinal de biodiversité des communautés de vers de terre à l'échelle de la France?

Une des rares règles générales a avoir été proposée sur les patrons de distribution spatiale de la diversité est que la diversité des communautés diminue de l'équateur vers les pôles (Stevens, 1989). Cette règle empirique semble se vérifier surtout à grande échelle (au moins 10 degrés de latitude), pour la majorité des organismes, à quelques exceptions près (Kraft *et al.*, 2011). Par ailleurs elle se retrouve également souvent à plus petite échelle. Chez lez organismes du sol cette règle a été peu testée, en particulier chez les vers de terre. Nous utilisons la base de Bouché pour tester cette règle.

Nous décomposons la biodiversité d'une zone en trois composantes complémentaires :

- La diversité totale, appelée diversité régionale "γ", qui correspond à la diversité de tous les organismes présents dans chaque région.
- La diversité au sein de chaque communauté, appelée diversité locale "α".
- La diversité "β", qui représente la différenciation entre communautés. Si β est élevé les communautés sont très différentes les unes des autres.

La taille de la région est généralement définie comme la zone maximale de mouvement d'un individu. Nous l'avons fixée à 150km dans cette étude, nous avons vérifié que les résultats restent robustes avec différentes tailles de régions.

La règle des gradients latitudinaux de diversité prédit que les diversités locales et régionales (α et γ) des vers de terre doivent diminuer du sud vers le nord de la France.

L'analyse des données de Bouché (Figure 3 a) montre que la diversité régionale (γ) des vers de terre diminue effectivement du sud vers le nord. En revanche, et de manière inattendue, la diversité locale α montre une tendance opposée nette, avec une augmentation vers le nord (Figure 3 c). La diversité β quand à elle diminue du sud au nord (Figure 3 b).

Les diversité γ et α des vers de terre varient donc de manière inverse avec la latitude en France.

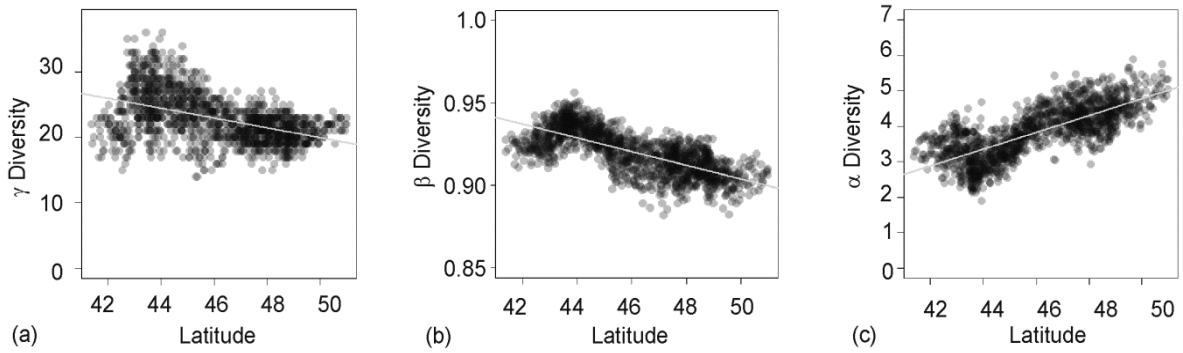
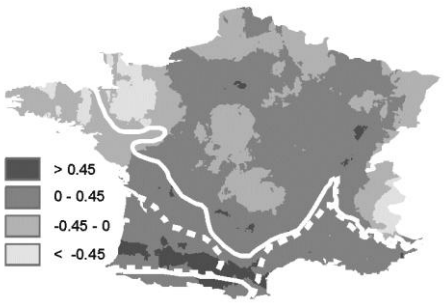


FIGURE 3 Diversité γ, β, α des vers de terre en fonction de la latitude en France (Mathieu & Davies, 2014).

Q°2 Y a-t-il des variations structurées des traits des espèces. En particulier y a-t-il un gradient latitudinal des traits des espèces?

Pour répondre à cette question nous avons regardé tout d'abord l'agrégation des traits, c'est-à-dire la variabilité des traits des espèces au sein des communautés. L'agrégation est nulle lorsque la variabilité locale des traits est égale à la variabilité générale, elle est positive lorsque les traits sont moins dispersés que la moyenne, et négative lorsque les traits sont plus variables qu'en moyenne. La Figure 4 montre clairement que les traits des vers de terre sont plus agrégés dans le nord que dans le sud. Il est souvent avancé que l'agrégation de traits résulte de processus de sélection par l'environnement, alors que la sur-dispersion des traits indique des processus d'évitement de la compétition. Selon cette assertion les communautés de vers dans le sud de la France seraient plus marquées par des processus de compétition alors que dans le nord de la France les communautés seraient constituées par des espèces ayant subi une forte sélection, comme par exemple sur le potentiel de dispersion. Cette interprétation est confortée par l'analyse de la composition des traits des communautés par RLQ, qui montre que l'axe principal de variabilité des traits des communautés est très corrélé avec la latitude (Figure 4).

A/ 

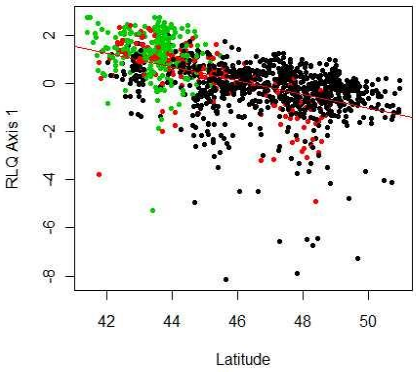
B/ 

FIGURE 4 A/ Agrégation des traits des communautés de vers de terre en France, B/ variabilité latitudinale des traits selon de l'axe 1 d'une RLQ sur les communautés de vers.

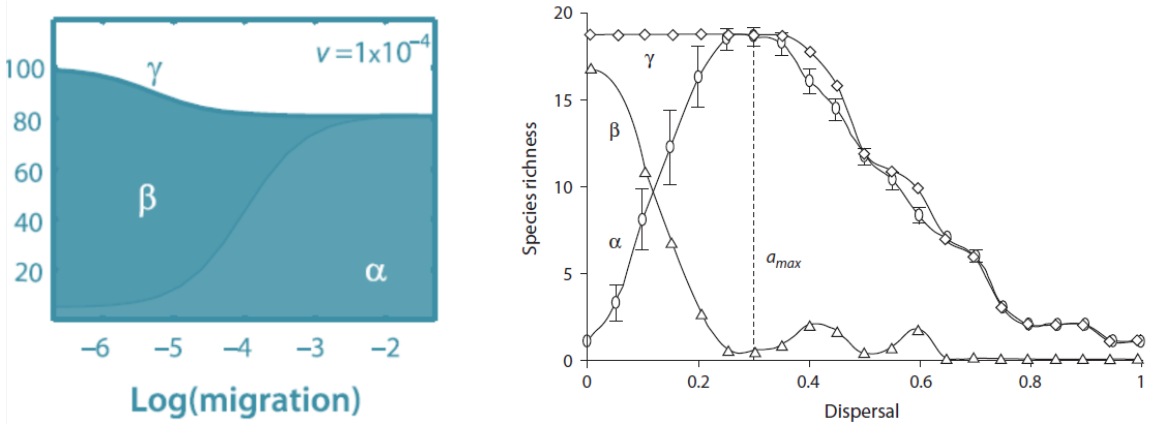
Q°3 Quels mécanismes ont abouti à la distribution actuelle de la biodiversité actuelle des vers de terre en France?

Afin de discuter des mécanismes qui ont pu générer la distribution spatiale des communautés de vers de terre en France, nous allons utiliser plusieurs approches de biogéographie et d'écologie des communautés qui proposent de faire le lien entre mécanismes et distribution.

Diversité α/γ

Les modèles théoriques montrent qu'il existe une relation entre le niveau de dispersion et le rapport entre la diversité locale et la diversité régionale α et γ.

Dans des communautés théoriques où la compétition est homogène (théorie neutre de la biodiversité), il n'y a pas d'exclusion compétitive systématique. Dans ce cas, lorsque la dispersion augmente, la diversité α augmente jusqu'à atteindre la valeur de γ (Figure 5 A/).



A/ B/

FIGURE 5 Diversité α, β γ, en fonction de l'intensité de dispersion dans une métacommunauté complètement connectée A) communauté neutre(Economo & Keitt, 2008. B) communauté non neutre (Mouquet & Loreau, 2003).

Dans les communautés où certaines espèces sont meilleures compétitrices que d'autres, la diversité α augmente aussi avec la dispersion jusqu'à atteindre la diversité γ , puis au-delà d'un certain seuil de dispersion a_{max}, α et γ diminuent et tendent vers 0 : la métacommunauté se comporte alors comme une seule communauté dans laquelle il y a progressivement exclusion compétitrice par les espèces les plus compétitrices.

Ce modèle suggère que la coexistence n'est possible que s'il n'existe pas dans les communautés des espèces qui sont à la fois très compétitives et à capacité de dispersion élevée, un point qui a été chaudement débattu (Yu & Wilson, 2001).

En résumé, quel que soit le postulat fait sur le rôle des interactions plurispécifiques, il apparaît que la structure des communautés dépend des mécanismes de dispersion. En particulier **lorsque la dispersion est faible, les diversités α et γ sont très différentes et le rapport α/γ est faible, alors que si la dispersion est forte, ces deux composantes sont proches et α/γ est élevé et tend vers 1.**

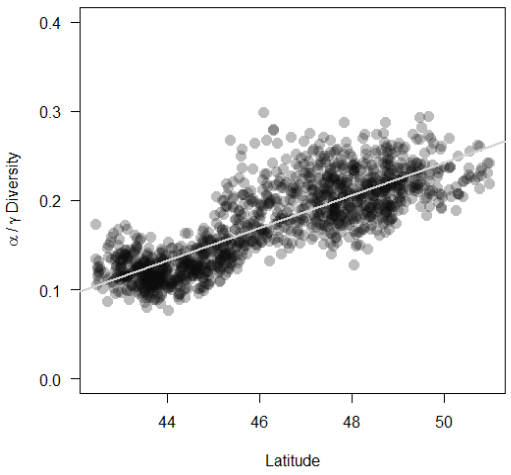
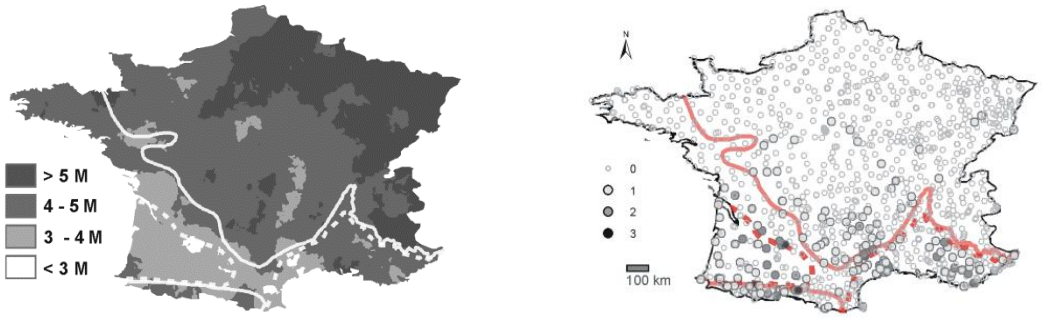


FIGURE 6 Diversité α sur γ des vers de terre en France en fonction de la latitude.

Dans le cas des communautés de vers de terre en France, le rapport α/γ augmente du sud vers le nord (Figure 6), ce qui suggère que la dispersion joue un rôle plus important dans la structuration des communautés dans le nord que dans le sud. Les espèces présentes dans le Nord seraient donc plus mobiles que les espèces présentes dans le sud. Cette hypothèse est appuyée par l'étude de l'aire de répartition des espèces. En effet on observe que les communautés du Nord – Nord Est sont composées par des espèces qui ont des aires de répartition en Europe plus larges que les espèces du Sud – Sud Ouest (Figure 7 A). Par ailleurs les espèces endémiques sont situées quasiment exclusivement dans le sud (Figure 7 B).



A/ B/

FIGURE 7 A/ Taille de l'aire de répartition des espèces de vers de terre en Europe (millions de km^2), B/ Carte du nombre d'espèces de vers de terre endémiques

Ce gradient latitudinal de composition des communautés peut résulter de plusieurs facteurs comme le climat actuel, le relief, mais être aussi le fruit de processus historiques, en particulier lié aux dernières glaciations. En effet, pendant les dernières grandes glaciations (LGM), le sol dans le nord du pays a été largement gelé (permafrost, indiqué par une ligne continue dans les figures précédentes) de sorte que la plupart des espèces de vers de terre ont alors probablement disparu dans le nord. Les rares zones non gelées, dans le sud de la France, ont probablement servit de refuge puis de source de recolonisation après la déglaciation. Les zones du nord de la France auraient été recolonisées uniquement par les espèces les plus mobiles (et théoriquement les

moins compétitrices), ce qui aurait généré le gradient longitudinal d'aire de distribution des espèces et du rapport α/γ. En faisant un calcul grossier, on peut considérer que les vers qui ont été capables de recoloniser le nord de la France depuis les refuges ont parcouru environ 800 km en 16000 ans, ce qui revient à parcourir au minimum 50 m par an. Ceci parait élevé mais possible (cf la deuxième partie du document pour des estimations quantitatives de la dispersion des vers de terre). L'examen des courbes latitudinales d'accumulation de biodiversité des vers de terre vient appuyer cette hypothèse.

En effet, si on considère une région dans laquelle les flux vont dans une direction privilégiée (Figure 8 A/), la diversité cumulée depuis la zone "source" vers la zone "puits" doit augmenter rapidement aux abords de la zone source, puis atteindre rapidement un plateau alors que l'on s'en éloigne. La diversité cumulée depuis la zone "puits" vers la zone "source" doit suivre un comportement différent, avec une faible augmentation du nombre d'espèces cumulé vers la zone puits, puis une forte augmentation en proximité de la zone source. L'endroit où se croisent les courbes indique le degré d'asymétrie entre zone source et zone puits. Si les flux se font de manière symétrique, les deux courbes doivent se croiser au milieu de l'espace considéré.

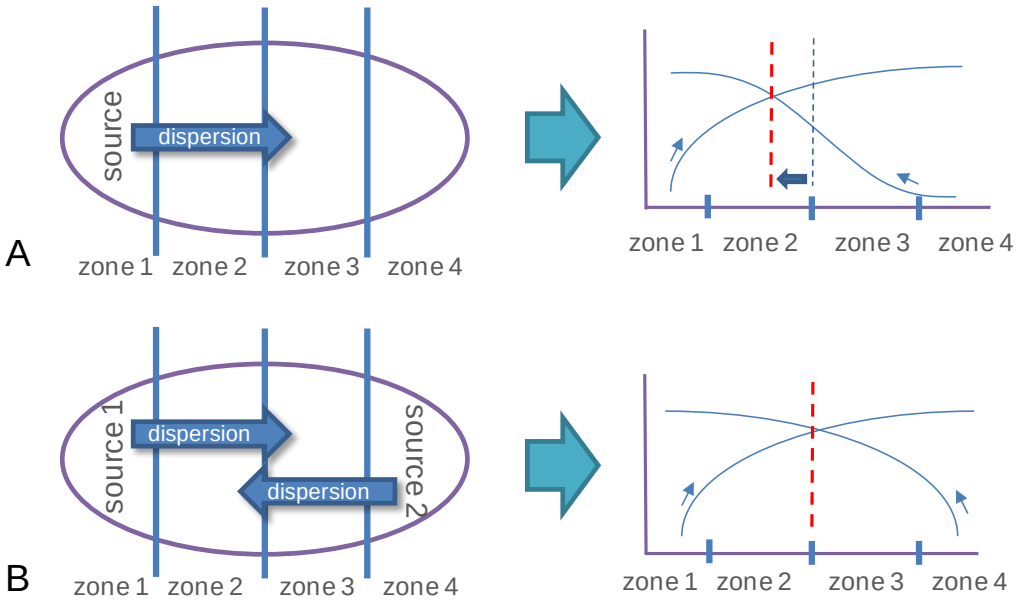


FIGURE 8 Forme de courbes d'accumulation en fonction de l'origine des flux. A) Les individus viennent exclusivement de la zone 1 (source). Les courbes s'interceptent vers la gauche. B) Les individus viennent indifféremment des zones 1 et 4. Les courbes s'interceptent à égale distance des zones sources.

La forme des courbes d'accumulation, et leur endroit d'intersection donne donc une indication sur la localisation des sources d'espèces. En France, si les zones refuges ont effectivement servi de source de recolonisation, on devrait avoir une intersection des courbes d'accumulation vers le sud.

C’est ce que l’on observe : les courbes d'accumulation des communautés de vers de terre se croisent nettement au sud (Figure 9), ce qui suggère à nouveau que les zones du sud de la France ont servi de source de recolonisation de la France.

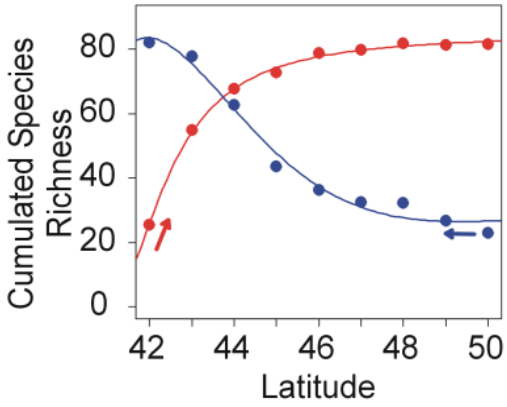


FIGURE 9 Courbes d'accumulation d'espèces de vers de terre en fonction de la latitude, en France. En bleu: courbe du nord vers le sud, en rouge : courbe du sud vers le nord

En recoupant ces informations, ainsi qu'en étudiant les traits des espèces, il apparaît que dans le sud de la France la diversité régionale γ est élevée, ce qui serait dû à son rôle historique de refuge. Dans le nord, seules certaines espèces ont pu recoloniser, si bien que la diversité régionale γ est plus faible. La diversité locale α est en revanche plus forte, soit parce que les espèces les plus dispersantes sont moins compétitives, comme cela a été supposé chez d'autres organismes, soit parce que l'exclusion compétitive n'a pas encore eu le temps d'opérer. Les espèces dans le nord sont plus mobiles et plus petites. Au final notre interprétation est que la capacité de dispersion a filtré les espèces suite aux déglaciations et créé un gradient de composition des communautés, et que la dispersion aurait joué un rôle limitant majeur dans la formation des communautés de vers de terre à l'échelle de la France.

1.2 DISPERSION DES VERS DE TERRE À L'ÉCHELLE DE LA RÉGION

A l'échelle de la région, peu d'études ont essayé de mettre en évidence le rôle de la dispersion dans la structuration des communautés de macrofaune du sol, en particulier chez les vers de terre. Le paradigme de l'écologie du paysage prédit que s'il y a des flux significatifs d'individus entre parcelles, et que si ces flux dépendent en partie des éléments composant le paysage, alors

- la différenciation génétique entre communautés est proportionnelle à la distance qui les sépare (Isolation By Distance : IBD).

- il existe un lien entre structure du paysage et structure génétique des populations (approche buffers).

Par ailleurs si la hiérarchie compétitive entre espèces ne joue pas un rôle déterminant dans la structuration des communautés (on parle de communautés neutres, concept développé dans le cadre du paradigme de la théorie neutre de la biodiversité UNTB Hubbel, 2001), alors on peut estimer le flux d'individus entre communautés grâce à l'équation d'Etienne (Etienne, 2005):

$$P[D|\theta, m, J] = \frac{J!}{\prod_{i=1}^{S} n_i \prod_{j=1}^{J} \Phi_j!} \frac{\theta^S}{(\theta)_J} \times \sum_{A=S}^{J} \left(K(D, A) \frac{(\theta)_J}{(\theta)_A} \frac{I^A}{(I)_J} \right)$$

où P[D/θ,m,J] représente la probabilité du jeu de données D (distribution des abondances des espèces) sachant θ, le paramètre fondamental de la diversité, m la probabilité d'immigration et J le nombre d'individus dans le pool. S représente le nombre d'espèces, Φ_j représente le nombre d'espèces d'abondance j, A représente le nombre d'ancêtres immigrants, I représente le nombre de migrants. la fonction K(D,A) est donnée par l'équation 5 dans Etienne, 2005. De plus, de manière générale :

$$(x)_y := \prod_{i=1}^{y} (x + i - 1)$$

A partir de ces équations il est possible d'estimer m et J par la méthode du maximum de vraisemblance (Etienne, 2005) à partir des données de terrain.

Estimer en situation réelle le paramètre m nécessite un important travail de terrain et de laboratoire, appuyé par un budget conséquent. Aussi, pour tester le rôle de la dispersion sur la formation des communautés de vers de terre, ai-je coordonné un projet ANR jeune chercheur (ANR JCJC "Edisp": Earthworm Dispersal), en collaboration avec plusieurs partenaires, l'U-PEC pour la génétique du paysage (Lise Dupont), l'Université de Rouen pour l'expertise sur les vers de terre (Thibaut Decaëns) et le MNHN pour l'imagerie aux Rayons X (Anick Abourachid). Le projet a permis de financer un post-doctorat en génétique des populations, Magally Torres, qui a travaillé avec Lise Dupont et moi même, et d'obtenir deux bourses de thèses: celle de Benoît Richard[1] sur les aspects spatiaux des communautés de vers à l'échelle de la parcelle, et celle de Gaël Caro[2] sur les mécanismes de dispersion chez les vers de terre. Ce dernier a publié plusieurs articles sur son travail de thèse et ses résultats sont présentés dans la partie "mécanismes de dispersion" de ce document. Les principaux résultats des travaux de terrain du projet EDISP sont présentés maintenant.

[1] Univ. de Rouen, décembre 2012, Directeur de thèse T. Decaëns
[2] UPMC, décembre 2012, Directeurs de thèse: T. Decaëns et moi même

Plan d'échantillonnage

Afin de tester le rôle de la dispersion sur la formation de communauté de vers de terre à l'échelle du paysage, nous avons développé un dispositif d'échantillonnage spatialisé multi échelles. L'aspect multi échelles s'est imposé car nous avions très peu de données sur l'échelle spatiale de dispersion des vers, et donc sur l'échelle spatiale à laquelle un signal pourrait être détecté. L'étude a été menée en Normandie, dans la région d'Yvetot, où les exploitations sont dominées par l'élevage, et comportent de nombreux pâturages, milieu dans lesquels les vers de terre sont abondants. D'après nos résultats sur les travaux de Bouché (chapitre précedent) les communautés de vers dans cette région sont caractérisées par des espèces relativement mobiles, ubiquistes, et peu structurées par la compétition.

A l'échelle de la région nous avons échantillonné 40 parcelles avec 5 blocs de sol de 25x25cm sur 20cm de profondeur, espacés de 10 m, poolés par parcelle pour les analyses. Parmi ces 40 parcelles, 3 parcelles ont fait l'objet d'une étude spatialisée beaucoup plus intense, avec un échantillonnage en grille de 100 points sur une maille régulière (Figure 10).

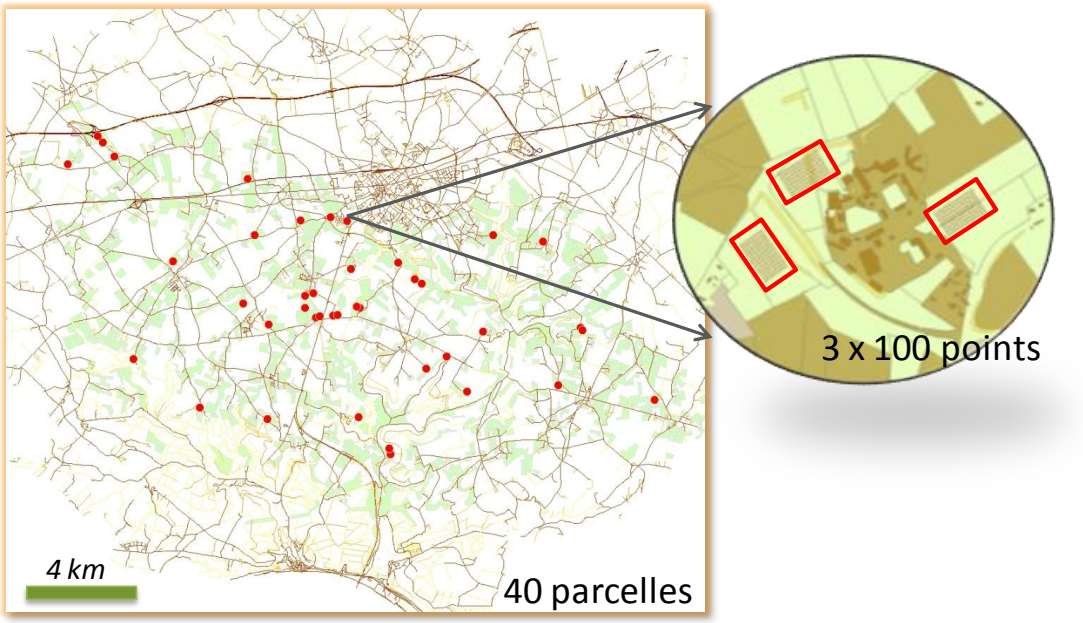


FIGURE 10 Plan d'échantillonnage des vers de terre en Normandie dans le projet ANR Edisp.

Nous avons sélectionné deux espèces abondantes à reproduction sexuée obligatoire pour l'étude génétique. Il s'agit de *Aporrectodea icterica* et *Allollobophora chlorotica* (Figure 11).

FIGURE 11 PHOTOS DE *A. ICTERICA* ET *A. CHLROROTICA* (MORPHE VERT)

Sur les grilles 141 individus de chaque espèce ont été recueillis, et 420 à l'échelle du paysage.

Résultats à l'échelle de la région

- **Isolation by Distance (IBD)**

Les résultats de l'approche IBD montrent que la différentiation génétique entre populations augmente très nettement avec la distance géographique chez *A.cholorotica* mais pas chez *A. icterica*, à l'échelle de la région (Figure 12, Annexe 2). Ceci suggère donc qu'il y a des flux limités d'individus chez l'espèce *A. chlrorotica* à cette échelle. L'étude à l'échelle de la parcelle, ainsi que les études expérimentales sur les mécanismes de dispersion suggère qu'à cette échelle la dispersion est probablement passive. Elle est sans doute assurée par un agent externe tel que des animaux ou les engins agricoles. Le manque de relation significative chez *A. icterica* peut être les résultats de plusieurs phénomènes. Soit il n'y a pas du tout de dispersion à cette échelle: les communautés sont complètement déconnectées les unes des autres. Soit au contraire elles sont tellement connectées qu'il y a homogénéisation génétique, et l'ensemble des communautés fonctionne comme une seule communauté. Soit enfin les marqueurs génétiques ne sont pas assez discriminants, si bien que l'on n'a pas à disposition les moyens de mettre en évidence la différentiation génétique.

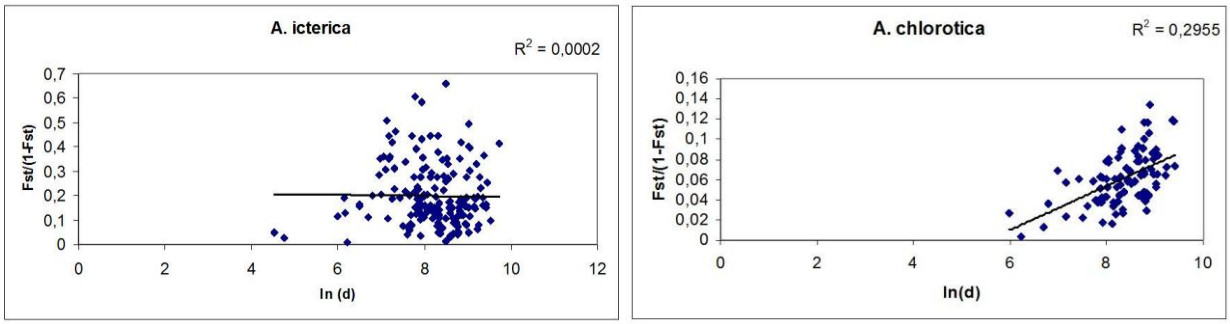


FIGURE 12 Différentiation génétique (a de Rousset) entre populations de *A. icterica* et *A chlorotica.* à l'échelle de la région en Normandie (tests de l'IBD).

- **Approche structure du paysage**

L'approche structure du paysage consiste à évaluer directement la relation entre la structure génétique et la structure du paysage avoisinant. La structure du paysage est décrite par une série d'indicateurs tels que la fragmentation, la taille des patchs d'habitats ou la distance inter patchs. (Figure 13 et Tableau 2)

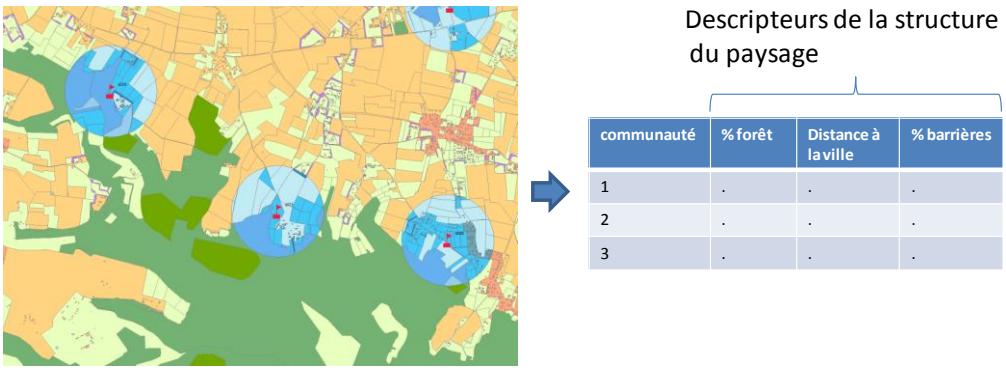


FIGURE 13 Approche typique en écologie du paysage: le paysage est décrit autour de chaque point d'échantillonnage dans un rayon donné, par des variables de structure paysagère.

	Aporectodea icterica				*Allolobophora chlorotica*		
	Fis	Ar	He		Fis	Ar	He
Anova table							
F	6.72	3.6	9.73		2.1	1.32	3.3
p - value	**0.003**	**0.03**	**<0.00**		0.2	0.51	0.18
adujsted r2	0.71	0.53	0.77		0.33	0.21	0.64
Predictors							
Patch density	ns	ns	ns		0.07	ns	ns
Edge density	-0.02	ns	ns		ns	ns	ns
Average patch area	-1.44	ns	ns		ns	ns	ns
SD of patch area	0.35	-0.007	-0.01		ns	ns	ns
Landscape diversity	7.4	ns	ns		ns	ns	-0.47
Patch Richness	-0.55	-0.009	ns		ns	ns	ns
Surface of reserves	0.4	ns	ns		ns	ns	ns
Surface of barriers	0.42	ns	ns		0.03	ns	-0.0002
Total ength of roads	ns	ns	-7.10-5		ns	ns	ns
Altitude	-0.02	ns	ns		ns	ns	0.0002

TABLEAU 2 Relation entre divers indices de structure génétique des populations (Fis : différentation génétique entre localités, Ar: Richesse allélique raréfiée, He: Hétérozigotie) et la structure du paysage avoisinant dans un rayon de 500m dans la région d'Yvetot en Normandie. Patch density : densité en patchs, edge densité : densité en lisières, Average Patch area: taille moyenne des patchs d'habitat, SD: écart type de la taille des patchs d'habitats, Patch Richness: diversité des types d'occupation du sol, Surface reserves: surfaces en zone réserves (favorables aux vers de terre), Surface of barriers : surface de zones barrières ne laissant pas passer les vers de terre, total length of road : longueur de route totale.

Les résultats montrent une forte corrélation entre la structure génétique des populations de *A. icterica* et la structure du paysage. En particulier les indices de différenciation génétique Fis et l'hétérozygotie (He) sont corrélés à plus de 70% à la structure du paysage. Les deux indices de structure génétique sont corrélés à la variabilité de la taille des éléments surfaciques du paysage. Le Fis est aussi corrélé à la plupart des indices de structure de paysage alors que He n'est corrélé qu'à la longueur de route. En revanche chez *A.chlorotica* il n'y a pas de relation significative avec la structure du paysage, bien que la variance expliquée soit relativement élevée.

Estimation de la probabilité d'émigration par la formule d'Etienne

Afin d'estimer une probabilité d'émigration depuis les différents sites, nous avons voulu utiliser l'équation d'Etienne, qui ne peut s'appliquer qu'aux communautés neutres, c'est-à-dire non dominées par une hiérarchie compétitive entre espèces. Afin de pouvoir mettre en œuvre cette approche il convient donc de vérifier le rôle de la compétition dans la formation des communautés. Ce point est complexe et plusieurs approches sont possibles, chacune présentant des avantages et des limites. Ici nous utiliserons l'indice du checkerboard, qui reflète le taux de paires d'espèces présentes de manière systématiquement exclusive : une seule des deux

espèces ne peut être présente dans la même communauté (cf. annexe 1 pour des détails sur la méthode), ce qui peut être considéré comme une évidence d'exclusion compétitive antérieure. L'indice obtenu, le C-score, est comparé à un modèle neutre considérant qu'il n'y a pas d'exclusion compétitive. Ceci permet d'évaluer si le C-score obtenu est significativement différent du C-score attendu sous l'hypothèse d'une communauté neutre. Dans le cas où les communautés semblent être peu façonnées par la compétition, la formule d'Etienne peut être utilisée pour calculer le taux de dispersion.

Dans la région considérée en Normandie la valeur médiane du C-score (0.45) n'est pas significativement différente de celle obtenue par un modèle neutre (0.44, p=0.67, Figure 14 A). Nous en déduisons qu'à cette échelle la hiérarchie compétitive ne joue pas un rôle évident dans la formation des communautés. Nous pouvons donc utiliser la formule d'Etienne pour calculer le taux de dispersion inter communautés "m".

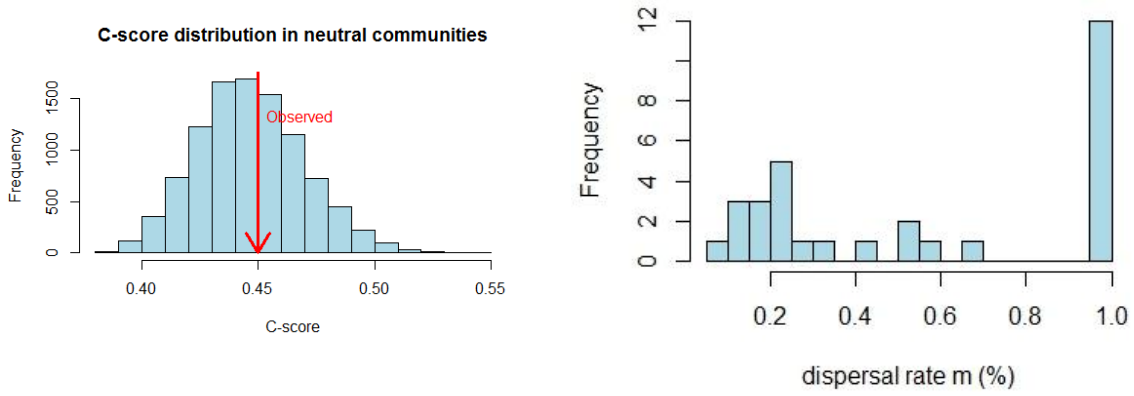


FIGURE 14 A) C-score observé et théorique sous l'hypothèse de neutralité, B) Histogramme des taux de dispersion inter communautés de vers de terre en Normandie.

La distribution des valeurs des taux de dispersion est bimodale, avec un pic autour de 0.2, et un autre pic sur l'intervalle 0.96-1 (Figure 14 B). Un seul site présente un taux de dispersion quasi nul, et aucun site ne présente de dispersion entre 0.7 et 0.9. Le pic 0.9-1 est probablement un artefact généré par la méthode d'estimation des paramètres et ne doit pas être pris en compte. Au final l'UNTB suggère la présence d'un niveau de dispersion intermédiaire non négligeable.

En résumé ces différentes approches à l'échelle de la région nous ont permis de mettre en évidence que :

- D'après l'équation d'Etienne il y a des flux non négligeables de vers de terre entre parcelles à l'échelle du paysage

- D'après les résultats de génétique des populations, les différentes espèces de vers dispersent de manière différente. En effet *A. chlorotica* montre une IBD significative mais pas *A. icterica*. Les résultats significatifs sur la structure du paysage suggèrent que les outils moléculaires disponibles sont assez fins pour détecter des différences chez *A. icterica*, et donc que l'absence d'IBD chez cette espèce serait plutôt due à de forts flux plutôt qu'à une limitation méthodologique par les outils moléculaires.

- Enfin il apparaît que les flux d'individus ne sont pas influencés par les mêmes facteurs chez les différentes espèces de vers de terre. *A. chlorotica* semble être dépendante de la distance inter-parcelles alors que *A. icterica* semble être influencée par la structure du paysage.

1.3 Dynamique de la biodiversité du sol et dispersion à l'échelle de l'exploitation après une perturbation

A une échelle plus fine la dispersion est plus à même de jouer un rôle déterminant dans l'assemblage des communautés car les distances à parcourir sont plus réduites. Le rôle de la dispersion peut être crucial en particulier dans les dynamiques de recolonisation après une perturbation majeure, et dans les dynamiques de recolonisation cyclique en milieu avec saisonnalité marquée.

Résilience des communautés après une perturbation majeure

Lorsqu'une communauté est soumise à une perturbation, sa résilience, entendue ici dans le sens de vitesse de retour à l'état initial, dépend de la capacité des espèces à recoloniser l'habitat. La résilience dépend ainsi fortement des capacités de dispersion des espèces depuis les zones refuges environnantes vers les zones perturbées. Ainsi la vitesse de résilience des communautés est un bon indicateur des capacités de dispersion des espèces. Quantifier la capacité de résilience des communautés revêt un enjeu capital en agroécologie où l'on veut que la biodiversité des organismes du sol puisse se régénérer naturellement après un événement entraînant une perturbation. Malheureusement peu de travaux ont tenté de mesurer la résilience de la macrofaune du sol après une perturbation. A contrario beaucoup d'études se sont attachées à faire le lien entre le degré d'intensification des pratiques agricoles et l'état des communautés. Dans cette dernière vision, les communautés du sol sont considérées comme des assemblages fixes, un peu à la manière de la phytosociologie. Au contraire, l'approche par la résilience considère les communautés comme des entités dynamiques, et prend naturellement en considération les mécanismes de formation des communautés, tels que la dispersion et les extinctions.

Dans cette optique je me suis intéressé à la résilience des communautés de la macrofaune du sol après une perturbation (cf. Annexe 3).

En Amazonie, dans les zones de déforestation, la forêt vierge est coupée afin de mettre en place des pâturages bovins (Figure 15). Il arrive cependant fréquemment que le pâturage ne soit pas mis en place et que les parcelles soient abandonnées. La nature reprend alors ses droits, ce qui fournit une occasion unique de mesurer la résilience des communautés, et apporte une première indication des capacités de dispersion de la macrofaune du sol.

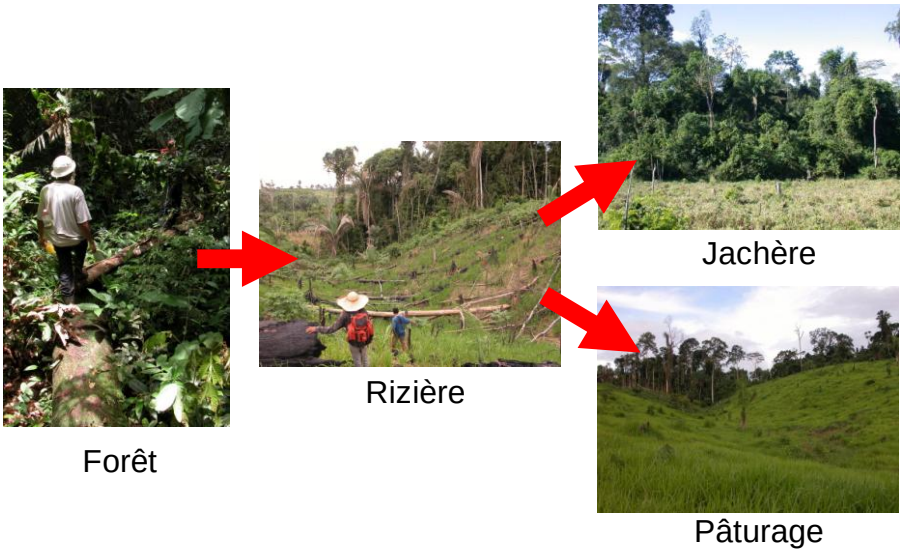


Figure 15 Itinéraire agricole typique après coupe de la forêt en Amazonie.

Nous avons ainsi pu voir que la coupe de la forêt avait un effet drastique sur la diversité de la macrofaune du sol (Figure 16). La diversité α, considérée ici comme la richesse spécifique par échantillon est divisée par trois (elle passe de 15 à 5 espèces par 1/16 de m^2) en quelques mois seulement après la coupe de la forêt (carré blanc puis noir Figure 16). Ceci illustre la sensibilité des communautés de macrofaune du sol aux conditions environnementales.

Lorsque les parcelles sont mises en pâturage, la biodiversité de la macrofaune du sol reprend faiblement (ronds noirs Figure 16). Par contre dans les parcelles en jachère la diversité reprend très vite et après 8 ans d'abandon elle a retrouvé une valeur proche de celle à l'origine (ronds blanc Figure 16). Cette tendance globale se retrouve chez la plupart des groupes de macrofaune du sol (Mathieu et al. 2005, Annexe 3). Ceci suggère une bonne résilience, et donc une certaine capacité à disperser, probablement de manière active depuis les parcelles adjacentes.

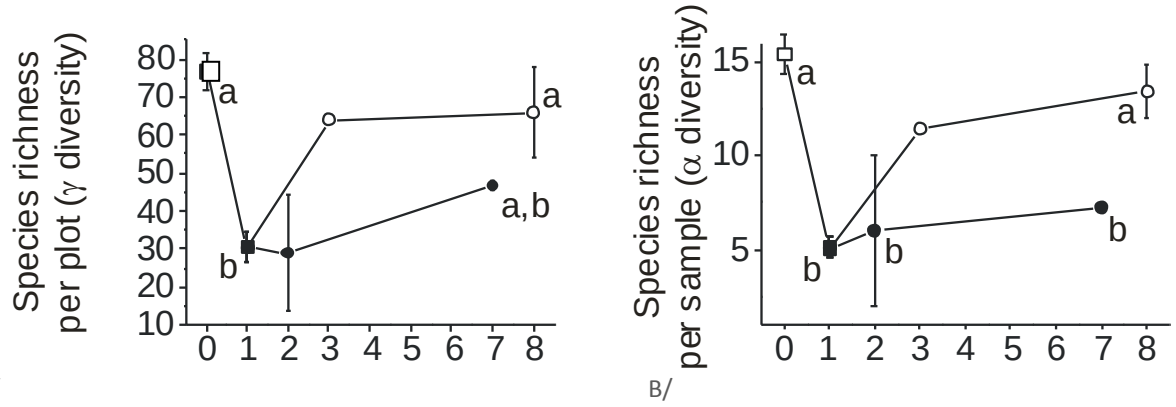


FIGURE 16 Diversité γ etα (Richesse spécifique A)par parcelle et B) par échantillon de 1/16m^2) de la macrofaune du sol total après la coupe de la forêt en Amazonie, Brésil (Carré blanc = forêt originale, carré noir = rizière temporaire, ronds noirs = pâturages, ronds blancs = jachère puis forêt secondaire) cf Annexe 3 pour les détails. Sur l'axe des x : nombre d'années après la coupe de la forêt.

Dynamique en milieu perturbé cycliquement

Une particularité des agrosystèmes exploités par rapport aux milieux naturels est la présence de fortes perturbations (récoltes, travail du sol, brulis) ayant lieu de manière régulière. D'après Wissinger (Wissinger, 1997, Figure 17), cette régularité exerce une pression de sélection sur les communautés qui va favoriser les espèces capables de disperser d'un habitat à l'autre pour éviter la perturbation temporaire. Une dynamique entre parcelles exploitées et parcelles au repos se met en place, et fonctionne comme une sorte de méta communauté, où les patchs sont temporaires. Dans ce contexte, le maintien de la diversité dans les parcelles exploitées nécessite qu'il y ait des zones refuges et que les individus soient capables de disperser entre patchs.

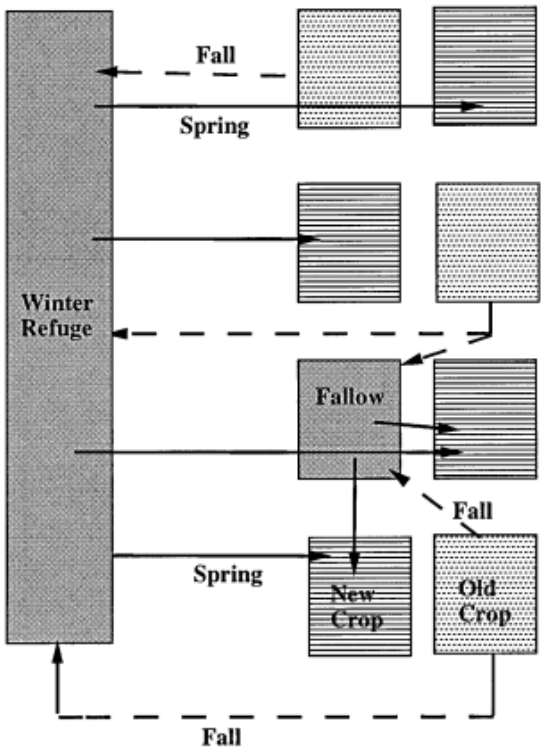


FIGURE 17 Modèle de colonisation cyclique d'un milieu agricole hétérogène d'après Wissinger, 1997.

Nous avons testé ce modèle sur les rizières en Thaïlande, où la mise en eau des parcelles (plots) constitue une perturbation majeure et régulière de l'environnement, alors que les digues (dykes) et les buttes (Mounds) séparant les parcelles représentent un habitat potentiel stable. Dans ce type de système les parcelles alternent entre deux états : inondées pendant la saison des pluies, et non inondées pendant la période sèche (Figure 18).

A

B

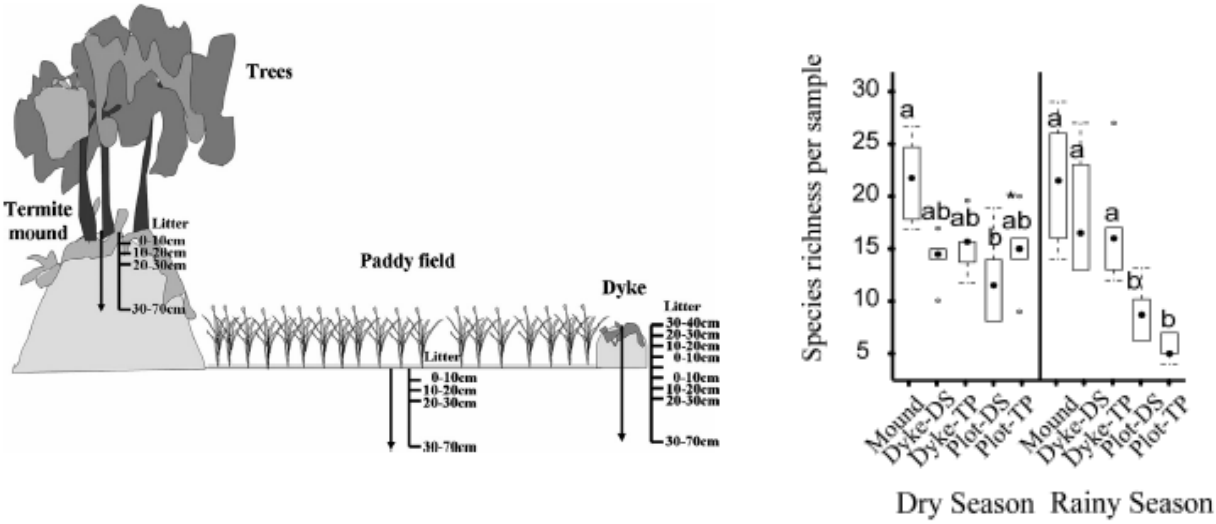
C

FIGURE 18 Photos de parcelles de rizières typiques en Thailande dans la région de la plaine de Khon Kaen. A) En saison sèche les parcelles sont asséchées. On distingue bien au centre une butte (Mound) et en bas à gauche une digue de séparation avec la parcelle adjacente. B) En saison humide les parcelles sont immergées. C) Dès que l'eau est à un niveau intermédiaire l'activité de la macrofaune du sol reprend entre les pieds de riz. (photos: P.Jouquet).

Les mises en eau constituent une contrainte très forte pour la macrofaune du sol qui ne peut se maintenir plusieurs mois sous l'eau. Malgré cela dès que le niveau d'eau baisse l'activité de la macrofaune du sol est visible au sein des parcelles. La question qui se pose est d'où viennent les organismes qui sont actifs au sein des parcelles temporairement inondées.

Chutinan Choosai, dans son travail de thèse[3] , mené en France et en Thaïlande, a traité cette question. Elle a testé le modèle de Wissinger selon lequel les parcelles joueraient le rôle de puits temporaires alors que les digues et les buttes joueraient un rôle de source. A l'occasion de ce travail la diversité de la macrofaune du sol en rizière a été déterminée dans les trois compartiments : en pleine parcelle, dans les digues et dans les buttes, en saison sèche et en saison des pluies (Figure 19 et cf. Annexe 4). Il a pu être montré qu'en saison humide, alors que les parcelles sont inondées, la diversité de la macrofaune du sol est faible dans les parcelles, mais élevée dans les digues et les buttes, alors que pendant la saison sèche, la diversité dans les parcelles atteint le niveau de diversité des digues, qui elle reste stable.

[3] Biological activity in paddy fields. The role of soil engineers in ecosystem functioning. 2010. Co-directeurs de thèse : C. Rouland, Y. Hanboonsong, Pascal Jouquet, co-encadrant : Jérôme Mathieu



a) b)

FIGURE 19 a) Schéma d'une rizière typique dans la région de Kon Kean (Thaïlande), et points d'échantillonnage de la macrofaune du sol. b) Diversité α (richesse spécifique) de la macrofaune du sol dans les différents endroits de la rizière en saison sèche et en saison des pluies. (cf. Annexe 4 pour les détails)

Cette alternance suggère que les digues et les buttes serviraient de refuge pendant la saison des pluies : en effet pendant la saison des pluies la macrofaune du sol est essentiellement concentrée dans les digues et les buttes. Lorsque la saison sèche arrive la macrofaune du sol des digues et des buttes migrerait vers les parcelles.

Ces résultats suggèrent que la dispersion est un mécanisme important pour l'établissement et le maintien des communautés de macrofaune du sol dans les rizières.

1.4 Dispersion à l'échelle de la parcelle

A l'échelle de la parcelle, les mouvements actifs des individus sont probablement la source majoritaire des déplacements de la macrofaune du sol. A cette échelle le terme dispersion est sujet à caution car il devient délicat de déterminer s'il s'agit de mouvements habituels, effectués à plusieurs occasions pendant la vie des organismes, ou s'il s'agit de véritable dispersion, c'est-à-dire de mouvements sans retour, impliquant généralement une certaine hétérogénéité de l'habitat, pas forcément significative à cette échelle. Afin d'aborder la question sous plusieurs angles, nous avons étudié les communautés de la macrofaune du sol dans plusieurs systèmes, présentant des niveaux d'hétérogénéité contrastés.

Dispersion en milieu peu structuré

En milieu peu structuré, du fait d'absence de limites claires de patchs d'habitats, il est difficile de déterminer comment est structurée la biodiversité. En particulier y a-t-il des limites aux communautés ou aux populations? Est-ce que la notion de communautés ou de populations locales a un sens dans ce contexte où les discontinuités environnementales sont peu claires? Peut-on définir des populations, et correspondent-elles à la structure spatiale des communautés?

Notre approche pour apporter des éléments de réponse à ces questions a consisté à faire des mesures directes de mouvements par capture recapture, et indirectes par la description de la distribution spatiale des variations génétiques et de la composition des communautés de vers de terre, en milieu homogène (prairies pâturées en Normandie). Ces travaux ont été principalement développés dans le cadre du projet ANR Edisp[4] que j'ai coordonné.

Approche capture recapture

Dans le cadre du projet Edisp[4], des expériences de capture marquage recapture ont été mises en place par Aurélie Husté de l'université de Rouen (Figure 20). Ces expériences ont montré que les vers se déplacent jusqu'à 2 mètres en 21 jours dans un milieu et une saison favorables.

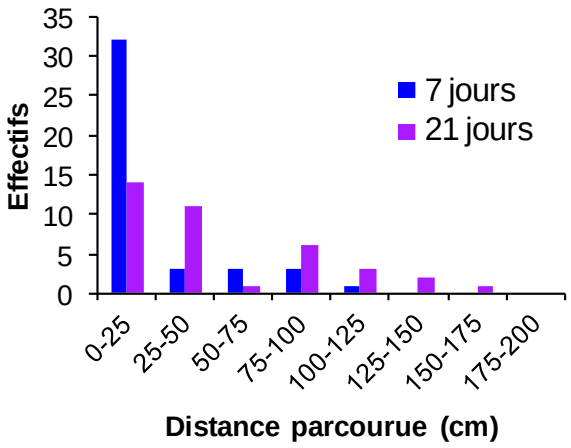


Figure 20 Distance de dispersion active des vers de terre sur 1 et 3 semaines mesurées dans le projet Edisp (Husté A. non publié).

[4] Projet ANR Jeunes Chercheur(e)s "Edisp" 2008-2012.

Approche génétique des populations

Une approche pour estimer le rôle de la dispersion est d'utiliser la génétique des populations avec un échantillonnage spatialisé intensif. Ceci a été mis en œuvre dans le projet Edisp sur deux parcelles de 8 x 12 points avec des grilles de 10m de maille (Figure 21, et Annexe 5).

En analysant le profil génétique des individus il est possible de tenter de déterminer les limites des populations, et également de tester à quelle échelle spatiale il y a isolement reproductif, et donc à partir de quelle échelle les individus ont peu de chance de disperser. On peut également mettre en œuvre une approche IBD comme mentionnée précédemment à l'échelle de la région. Cette approche a été réalisée en collaboration avec l'équipe de Lise Dupont (Upec) dans le cadre du projet Edisp que j'ai coordonné. Nous avons également développé une approche Isolation by Resistance (IBR), qui consiste à déterminer le chemin de moindre coût de déplacement entre sites afin de calculer une distance 'fonctionnelle" entre sites. Pour cette approche, les coûts ont été calculés à partir de la résistivité du sol, qui dépend de la structure du sol, et reflète son fonctionnement hydrique. Cet indicateur des propriétés du sol est connu pour être corrélé avec la densité en vers. Plusieurs scénarios de coûts ont été testés, en particulier nous avons testé les hypothèses de corrélation positive, négative et d'écart à la moyenne de résistivité entre densité en vers et résistivité du sol.

FIGURE 21 Vue aérienne des points d'échantillonnage spatialisé en grille sur les deux parcelles utilisées pour la génétique des vers de terre dans le projet Edisp en Normandie.

Les résultats montrent que chez *A.chlorotica* on peut délimiter des clusters génétiques dans une des parcelles (Figure 22), et qu'il y a un IBD significatif. Ceci nous a permis d'estimer l'existence d'un flux de 7.6m par an chez cette espèce, en utilisant le a de Rousset. En revanche chez *A.icterica* pas d'IBD significatif n'a été trouvé, et pas de clusters génétique non plus. Cette absence de résultat peut être interprétée soit comme un manque de

sensibilité des marqueurs moléculaires, soit comme l'existence de nombreux mouvements d'individus de *A. icterica* à cette échelle. Les tests de Mantel suggèrent également que les propriétés du sol jouent un rôle significatif sur la structuration spatiale génétique, l'IBR étant significative dans la plupart des cas (Tableau 3).

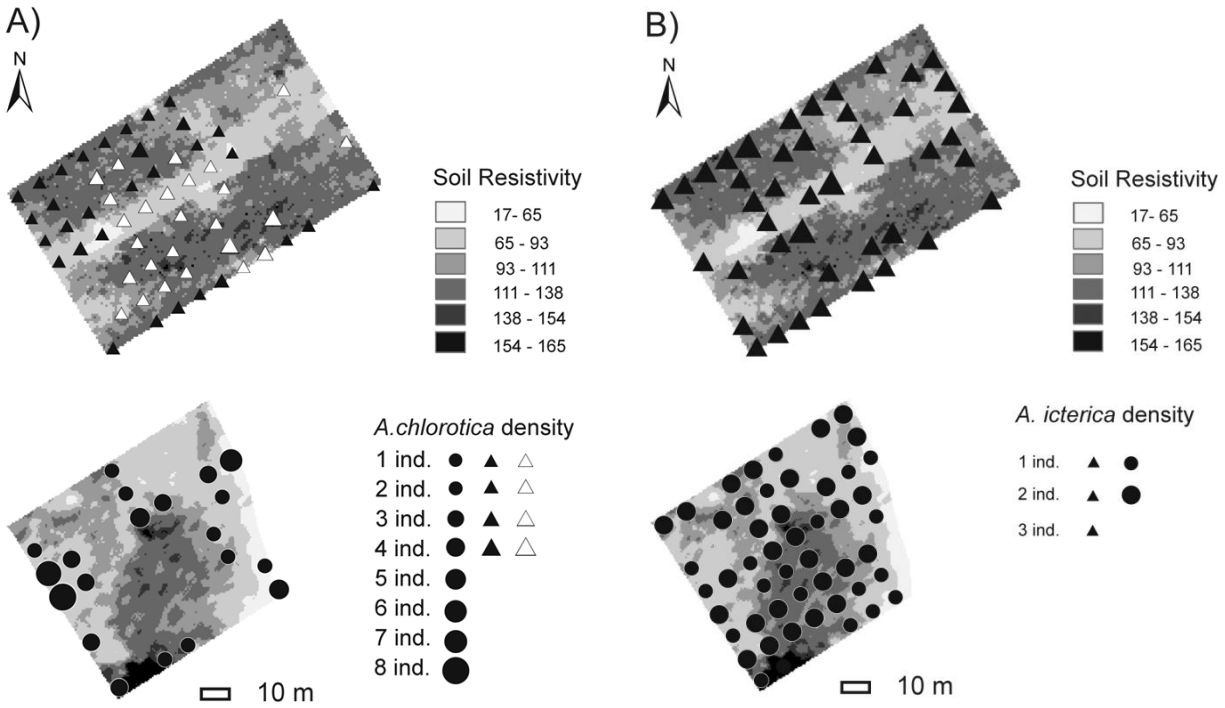


FIGURE 22 A) Densité de *A. chlorotica* dans deux parcelles, et cluster génétique d'appartenance, B) idem pour l'espèce *A. icterica*. Le fond de la carte indique la résistivité du sol.

TABLEAU 3 Résultats des tests de Mantel partiels pour détecter de l'isolation par la distance (IBD) ou de l'isolation par resistance (IBR). D = distance geographique, R = distance basée sur la résistivité du sol et R*= résititivité moyenne, 1/R = distance basée sur l'inverse de la résitivité, |R*-R|= distance basée sur l'écart à la résistivité moyenne du sol. La formule X1/X2 signifie l'effet de X1 alors que l'effet de X2 est contrôlé.

Species	Plot	Predictor	Mantel r	p-value		IBD or IBR
A. chlorotica	P_A	D	-0.04	0.002	*	
		R	-0.07	<10-4	*	
		D/R	0.1	<10-4	*	IBD
		R/D	-0.12	<10-4	*	
		1/R	-0.03	0.04	*	
		D/(1/R)	-0.1	<10-4	*	
		(1/R)/D	0.1	<10-4	*	IBR
		|R-R*|	-0.03	0.01	*	
		D/|R-R*|	-0.03	0.03	*	
		|R-R*|/D	0.003	0.4	ns	
A. chlorotica	P_B	D	0.12	<10-4	*	IBD
		R	0.13	1.10-4	*	IBR
		D/R	-0.03	0.14	ns	
		R/D	0.06	0.028	*	IBR
		1/R	0.11	<10-4	*	IBR
		D/(1/R)	0.04	0.08	ns	
		(1/R)/D	-0.02	0.3	ns	
		|R-R*|	0.08	0.004	*	IBR
		D/|R-R*|	0.09	0.002	*	IBD
		|R-R*|/D	-0.015	0.3	ns	
A. icterica	P_A	D	0.02	0.17	ns	
		R	0.04	0.04	*	IBR
		D/R	0.014	0.27	ns	
		R/D	0.09	0.34	ns	
		1/R	0.05	0.02	*	IBR
		D/(1/R)	0.03	0.06	ns	
		(1/R)/D	-0.03	0.08	ns	
		|R-R*|	0.05	0.02	*	IBR
		D/|R-R*|	-0.01	0.3	ns	
		|R-R*|/D	0.03	0.08	ns	
A. icterica	P_B	D	-0.05	0.001	*	
		R	-0.06	<10-4	*	
		D/R	-0.019	0.13	ns	
		R/D	0.003	0.44	ns	
		1/R	-0.05	0.001	*	
		D/(1/R)	-0.02	0.19	ns	
		(1/R)/D	0.002	0.43	ns	
		|R-R*|	-0.06	<10-4	*	
		D/|R-R*|	-0.02	0.16	*	
		|R-R*|/D	-0.04	0.012	*	

Au final nous pouvons tirer plusieurs conclusions sur la dispersion des vers de terre à l'échelle de la parcelle :

- La dispersion active semble limitée à 28 mètres par an.
- La distance de dispersion ne semble pas être différente selon l'âge des individus et leur catégorie écologique.
- Les espèces semblent avoir des patterns de dispersion qui leur sont propres (ici *A. icterica* semble plus mobile que *A. chlorotica*).
- Chez certaines espèces il peut y avoir un isolement reproductif à l'échelle de la parcelle, autrement dit la dispersion peut être limitante à cette échelle.
- Les propriétés des sols semblent influencer les mouvements d'individus, en particulier chez certaines espèces (*A. icterica* ici).

1.5 Mouvements à l'échelle micro-locale

Certains milieux comme les pâturages Amazoniens sont extrêmement hétérogènes à petite échelle, par exemple des surfaces de l'ordre de 10m^2. En particulier les touffes d'herbes sont clairement limitées et séparées spatialement par du sol nu. Cette hétérogénéité du couvert végétal structure très fortement la diversité de la macrofaune du sol (Figure 23, Annexe 6). La densité et la diversité de la macrofaune du sol sont deux fois plus élevées sous les touffes d'herbes que dans le sol nu.

A/ 

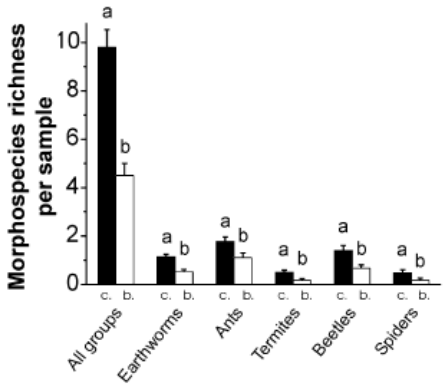
B/ 

Figure 23 A) Aspect d'un pâturage typique planté en *Bracharia bryzantha* en Amazonie. On voit bien la structuration du milieu avec les touffes d'herbes séparées par du sol nu B) Diversité de la macrofaune du sol sous les touffes d'herbe a) et à côté b).

Dans ce milieu la diversité est donc très localisée dans des patchs d'habitat favorables (les touffes d'herbes) séparés par une matrice défavorable. On peut alors se demander quels mécanismes permettent le maintien de la biodiversité de la macrofaune du sol entre les touffes d'herbes, qui sont des zones défavorables. Une possibilité est que ces zones fonctionnent comme un système sources-puits, où la biodiversité dans les puits (les zones défavorables, c.a.d. le sol nu) serait maintenue par l'apport d'individus depuis les zones sources (les zones d'habitat favorables, cad les touffes). Dans un tel système la biodiversité des zones défavorables devrait donc dépendre de la diversité qui arrive depuis les zones sources. Cet apport dépend du niveau de diversité dans les zones sources, qui dépend elle-même de la configuration spatiale des zones sources. La configuration spatiale des zones puits devrait donc influencer à la fois la diversité dans les zones sources et puits.

Pour tester cette hypothèse nous avons échantillonné 60 zones de 3mx3m de pâturages en *Brachiaria bryzantha* en Amazonie (Ben Fica, Para), dans lesquelles nous avons prélevé la macrofaune du sol dans un échantillon en zone source et un en zone puits. La distribution spatiale des touffes d'herbes a été cartographiée (Figure 24) afin de calculer des indicateurs de configuration spatiale des zones sources (AERA: surface totale des patchs, PA: Taille moyenne des patchs., ED : densité de bordure, PD: densité en patchs, LPI : taille du plus grand patch "source", Dist : distance au patch source le plus proche). Ensuite la relation entre la biodiversité de la macrofaune du sol (densité et richesse spécifique) a été évaluée par régression multiple.

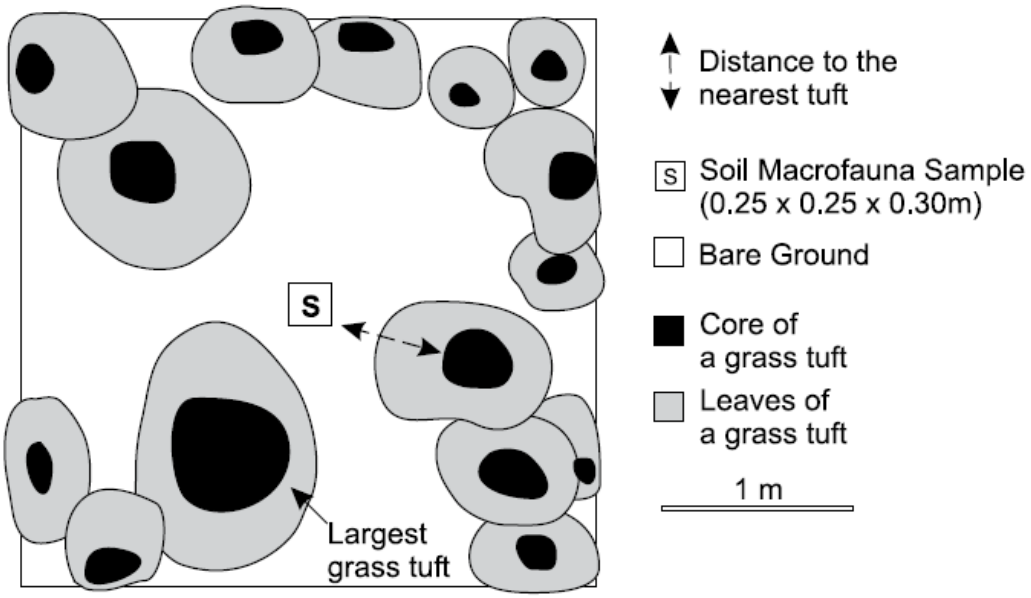


FIGURE 24 Réprésentation schématique du couvert végétal dans un pâturage amazonien planté en *Brachiaria bryzoides*, dans la région de Maraba, Para.

Les résultats montrent que la biodiversité dans les zones puits et sources est corrélée avec la configuration spatiale des zones sources, en particulier la distance à la zone source la plus proche, la surface occupée par les zones sources, et la taille de la plus grosse zone source (Tableau 4). Ce résultat suggère qu'à cette échelle aussi les dynamiques liées au mouvement des individus jouent un rôle dans le maintien de la diversité locale de la macrofaune du sol.

Group	Dependant variable	Sample type	Coefficients of the linear model
earthworms			
	Species Richness (ln) $r^2aj = 0.33$	Bare Ground Microsite	$0.51 + 0.16 \times LPI - 0.14 \times PD$ $1.02 + 0.01 \times LPI - 0.14 \times PD$
	Density (ln) $r^2aj = 0.38$	Bare Ground Microsite	$0.78 - 0.22 \times PD$ $1.73 - 0.22 \times PD$
All together			
	Species Richness (ln) $r^2aj = 0.68$	Bare Ground Microsite	$1.57 - 0.48 \times DIST + 0.17 \times AREA - 0.29 \times ED$ $1.94 - 0.48 \times DIST + 0.17 \times AREA + 0.01 \times ED$
	Density (ln) $r^2aj = 0.69$	Bare Ground Microsite	$2.18 - 0.60 \times DIST + 0.45 \times LPI - 0.19 \times ED$ $3.15 - 0.60 \times DIST + 0.45 \times LPI + 0.33 \times ED$

TABLEAU 4 Résumé des régressions multiples entre la richesse spécifique, la densité des vers de terre et de la macrofaune du sol totale, dans le sol nu (Bare Ground) et sous les touffes d'herbe (Microsite) et les indices de configuration spatiale des patchs de touffes d'herbe. LPI : taille de la plus grosse touffe d'herbe, DIST: distance à la touffe d'herbe la plus proche, AREA : Surface totale en touffe d'herbe, ED: densité en bordure de touffe d'herbe.

2. MÉCANISMES DE DISPERSION CHEZ LES VERS DE TERRE

Comme nous l'avons vu précédemment, la dispersion est un trait d'histoire de vie central qui affecte les processus de formation et de maintien des communautés, la colonisation de nouveaux habitats et les flux génétiques. Comprendre les déterminismes de la dispersion revêt donc un enjeu capital pour la compréhension du vivant. Les mécanismes de dispersion chez les organismes du sol ont été relativement peu étudiés (Tableau 1 p.9), en regard de leur rôle écologique et évolutif. En particulier extrêmement peu d'études ont été menées sur les mécanismes de dispersion des vers de terre, alors que c'est un des organismes les plus abondants et des plus influant sur le fonctionnement des écosystèmes tempérés. Afin de combler ce manque, j'ai encadré un travail de doctorat (Gael Caro, 2009-2012[5]) dans le cadre du projet ANR Edisp, sur les déterminismes de la dispersion chez les vers de terre. Les principaux résultats –majoritairement issus de la thèse de G. Caro- sont exposés ci-après.

De manière générale on peut classiquement distinguer deux grands types de déterminants de la dispersion : les facteurs intrinsèques aux individus – c'est-à-dire liés au génotype ou au phénotype de l'individu- et les facteurs externes, ou environnementaux. Parmi les déterminants intrinsèques, on peut citer l'âge, le statut hormonal et la taille corporelle. Ces différents déterminants intrinsèques peuvent être interdépendants de manière antagoniste, de sorte que l'aptitude à disperser est le fruit d'un compromis entre différents traits d'histoire de vie. Les déterminants externes font référence par exemple à la qualité de l'habitat, la quantité de ressources ou la densité intraspécifique. Chez les vers de terre, aucun de ces déterminants -internes ou externes- n'avait été étudié avant le projet Edisp.

La dispersion peut être décomposée en trois étapes distinctes. La première étape est le départ du lieu initial vers une destination non encore connue par l'individu. La seconde étape est le déplacement vers le lieu d'arrivée. La dernière étape est l'établissement dans le lieu d'arrivée, qui peut s'assimiler à de la sélection de l'habitat. Chaque étape engendre des coûts et des bénéfices potentiels, et ont des déterminants potentiellement différents. Un même facteur peut avoir un effet différent sur les différentes étapes de la dispersion. Dans le projet Edisp nous avons essayé de dresser les grandes lignes de ces déterminants pour les étapes 1 et 2.

FIGURE 25 Les 3 étapes de la dispersion

[5]UPMC, décembre 2012, Directeurs de thèse: T. Decaëns et moi même

2.1 Déterminants du déclenchement de la dispersion

Un principe général qui a été dégagé est que la dispersion augmente lorsque les coûts de dispersion sont perçus comme étant inférieurs aux bénéfices obtenus en quittant le patch. La plupart des espèces estiment correctement la qualité de l'habitat et dispersent davantage depuis des habitats de faible qualité que depuis des habitats de bonne qualité. Il a été également montré que certaines espèces ne sont pas toujours capables d'évaluer correctement la qualité de l'habitat et vont par exemple être attirées par un milieu non propice à leur reproduction. Chez les vers le comportement face à un habitat défavorable dans un environnement couteux pour la dispersion n'avait pas été évalué. La dispersion chez les vers revêt a priori des coûts très élevé, liés à leur lenteur de déplacement et à leur dépendance aux conditions microclimatiques, ce qui rend fortement hasardeux les déplacements. De sorte qu'il n'est pas évident que les vers répondent par la dispersion à la présence d'un habitat défavorable.

Qualité de l'habitat

Nous avons mené une série d'expériences en milieu contrôlé afin de tester le rôle de la qualité de l'habitat sur la propension à disperser chez les vers. (Mathieu *et al.*, 2010, Caro *et al.*, 2013). Nous avons trouvé que toutes les espèces de vers étudiées étaient très réactives à la qualité de l'habitat, et dispersaient plus fortement depuis les habitats inhospitaliers (Figure 26 et Annexes 7&8).

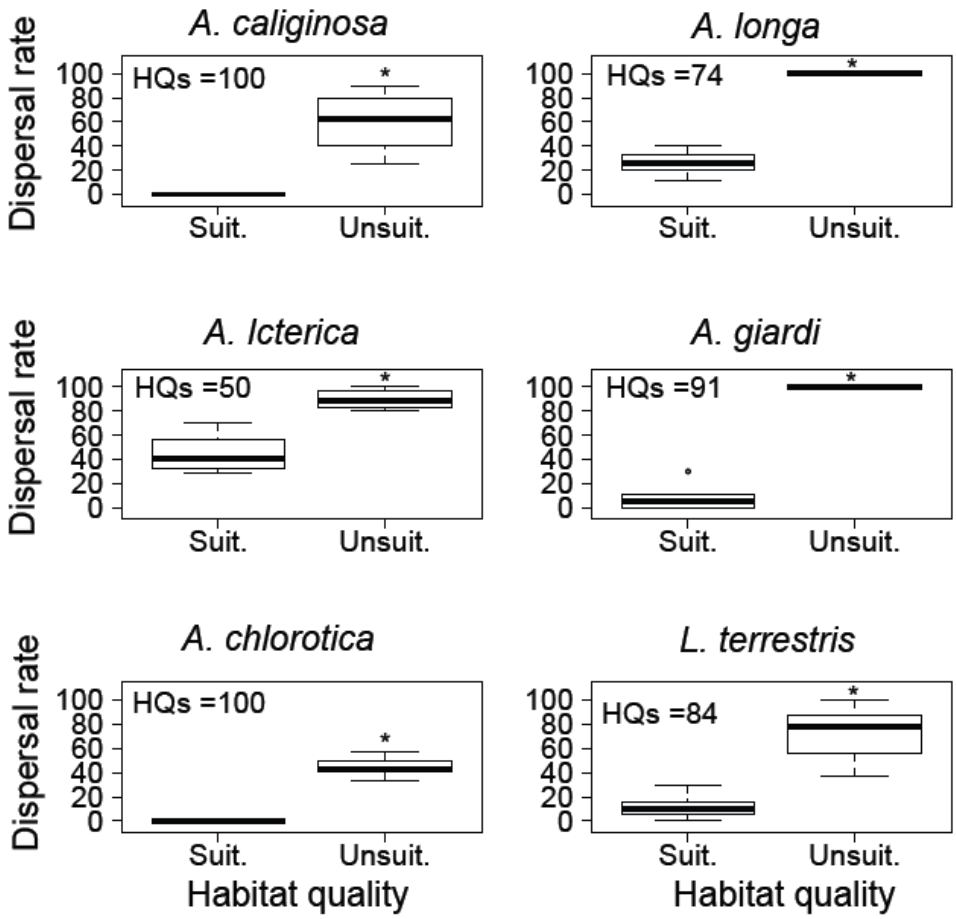


Figure 26 Taux de dispersion en fonction de la qualité de l'habitat de départ pour différentes espèces (colonne de gauche : endogées, colonne de droite : anécique). Suit. = habitat favorable, Unsuit. : habitat défavorable.

Nous avons également mis en évidence que les vers épigés évaluaient la qualité de l'habitat non seulement d'un point de vue quantité de ressources alimentaires, mais aussi du point de vue physique de l'habitat,

indépendamment de l'aspect ressources. En effet les vers de l'espèce *Dendrobena veneta* dispersent moins depuis un milieu avec une litière artificielle non comestible, simulant une vraie litière, que depuis un milieu sans litière (Figure 27, annexe 8).

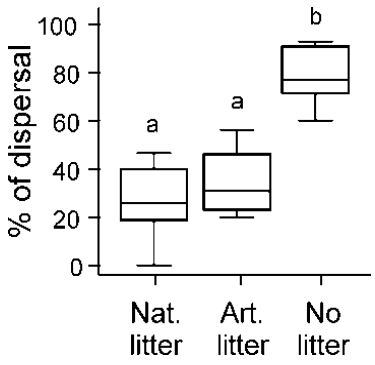


FIGURE 27 Dispersion de *D. veneta* depuis un milieu avec litière naturelle (Nat. litter), avec litière artificielle (Art. litter) et sans litière (No litter).

DENSITÉ DÉPENDANCE

Les résultats précédents montrent que les vers réagissent en dispersant lorsqu'ils sont exposés à des conditions défavorables. Ce comportement permet de faire le lien entre les propriétés du milieu et la perception qu'en ont les vers: si les vers dispersent fortement d'un milieu, c'est qu'ils le jugent défavorable.

Nous sommes partis de cette constatation pour explorer les capacités des vers à détecter et réagir à la densité en congénères. De nombreuses études ont montré que les taux de reproduction et de croissances des vers de terre étaient très liés à la densité intraspécifique, ce qui suggère une certaine sensibilité à la densité. D'un autre côté, les vers de terre sont souvent distribués spatialement en patchs de forte densité, espacés par des zones peu peuplées, ce qui suggère une certaine forme de tolérance à la densité intraspécifique. D'un point de vue théorique, il est classiquement admis que l'augmentation de la densité intraspécifique va entraîner une pression sur les ressources et aboutir à de la dispersion.

Nous avons testé ce postulat sur six espèces, en introduisant des densités croissantes d'individus dans des dispositifs de dispersion (Figure 28 et Annexe 7). Nous avons trouvé des grandes disparités de réponse entre espèces. Toutes les espèces anéciques sont très sensibles à la densité, alors que la majorité des espèces endogées testées sont insensibles à la densité intraspécifique.

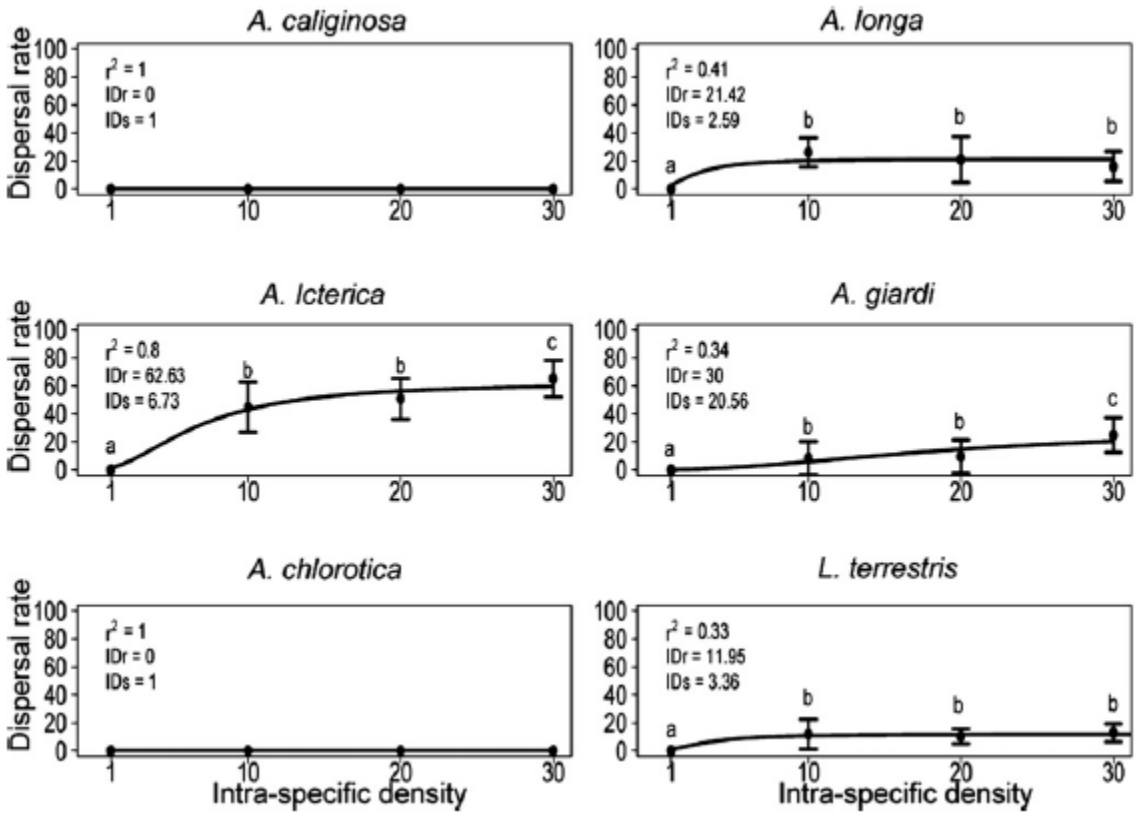


FIGURE 28 Dispersion en fonction de la densité chez trois espèces endogées (colonne de gauche) et trois espèces anéciques (colonne de droite).

FEEDBACK INGÉNIÉRIE ÉCOLOGIQUE – DÉCLENCHEMENT DE LA DISPERSION

La relative faible sensibilité des espèces endogées à la densité intraspécifique est assez surprenante et questionne sur la perception du lien entre densité intraspécique et qualité du milieu chez les vers de terre, au moins chez les endogés. Une composante forte du comportement des vers endogés est leur activité de creusement des galeries, qui modifie les propriétés de leur habitat. Cette action des vers sur leur habitat peut potentiellement entraîner une boucle de rétroaction positive entre densité et qualité de l'habitat, qui se répercuterait sur l'inclinaison à disperser. Afin de tester l'existence d'une telle boucle de rétroaction, nous avons mené une série d'expériences.

En premier lieu nous avons essayé d'évaluer la perception par les vers d'un milieu utilisé récemment par des congénères par rapport à un sol vierge. Pour cela nous avons comparé la tendance à disperser depuis un milieu vierge à celle depuis un milieu utilisé récemment par des congénères. Deux issues étaient possibles :

- soit, les vers perçoivent le milieu pré utilisé comme étant appauvri, car des ressources ont été consommées, ou comme étant "souillé", à cause de l'accumulation de déjections nuisibles, et dans ce cas les vers disperseraient d'avantage que depuis un milieu vierge. C'est le seul cas de figure prévu par la théorie de la dispersion jusqu'ici.

- soit les vers perçoivent la pré utilisation du sol comme une amélioration dans la qualité du milieu, par exemple due à la préexistence de galeries qui faciliteraient les déplacements dans le sol et dans ce cas les vers disperseraient moins depuis un milieu pré utilisé que depuis un milieu vierge.

Les résultats montrent que les vers dispersent moins depuis un milieu pré utilisé par les congénères que depuis un sol "vierge" (Figure 29 et Annexe 8). Ceci suggère donc une boucle de rétroaction positive, au moins à court terme, entre activité des vers et perception de la qualité du milieu, avec comme conséquence une réduction de la dispersion depuis les milieux pré utilisés.

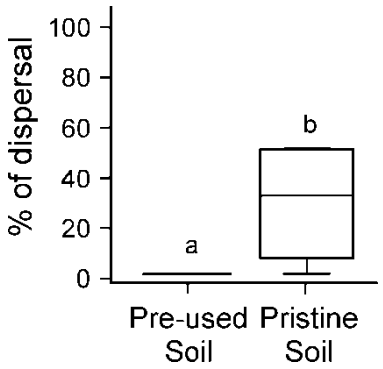


FIGURE 29 Dispersion de *Aporrectoda icterica* depuis un sol utilisé récemment par des congénères (Pre-used Soil) et depuis un sol "vierge", non utilisé récemment par des congénères.

Cette boucle de rétraction positive à court terme se comprend bien si on considère le fait que les vers de terre aménagent leur milieu. Cependant on peut s'interroger sur l'effet à long terme de l'exploitation du milieu par les congénères. En effet au-delà d'un seuil d'utilisation cumulée, le milieu peut devenir trop transformé et devenir moins attractif pour les vers. Dans ce cas l'attractivité du milieu commencerait à augmenter puis diminuerait avec l'utilisation cumulée du milieu (Figure 30). En conséquence la dispersion depuis ce milieu devrait être initialement plus faible que depuis un milieu vierge, diminuer jusqu'à un minimum puis ré-augmenter jusqu'au niveau initial, voir le dépasser.

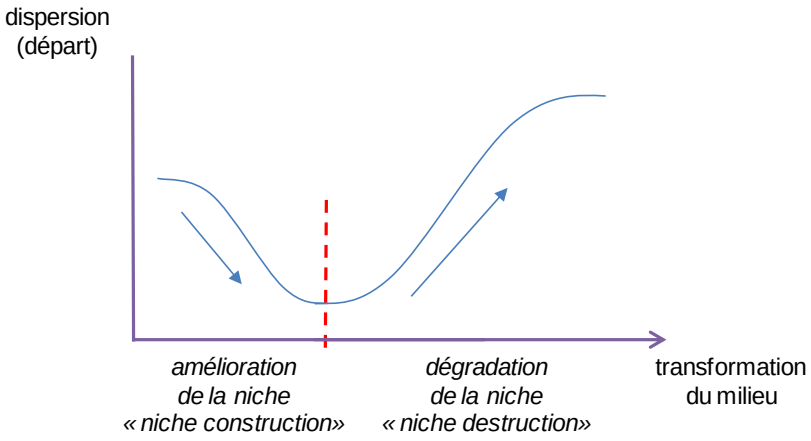


FIGURE 30 Boucle de rétroaction hypothétique entre utilisation cumulée du milieu et dispersion. A faible utilisation cumulée du milieu les vers améliorent le milieu et donc dispersent peu du milieu. Au-delà d'un certain seuil d'utilisation le milieu est trop transformé et les vers dispersent.

Afin de tester ce scénario nous avons introduit des vers dans des sols pré utilisés sur des périodes croissantes (0, 1, 2, 4, 6 semaines). Nous avons décrit les variations physico chimiques du milieu associées à cette utilisation, puis nous avons fait le lien entre le degré de transformation du milieu et la dispersion.

Les résultats (Annexe 9) montrent qu'au cours de l'expérience le milieu a été fortement transformé physiquement par les vers de terre mais peu transformé chimiquement. En particulier le sol a été compacté localement entre les galeries, et décompacté au sein des galeries. En résumé les vers ont augmenté l'hétérogénéité du sol. La mesure de la "force" du sol traduit ce changement et se mesure en mesurant la résistance à la pénétration du sol par une tige fine (Figure 31).

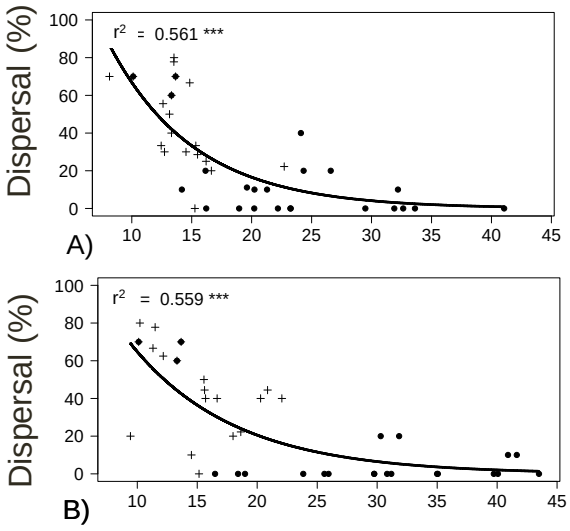


FIGURE 31 Dispersion d'*Aporrectodea icterica* en fonction du taux de modification physique du milieu par lui-même (en haut)et par *A. giardi (en bas)*. La modification du sol est mesurée par la "force" du sol, qui traduit l'hétérogénéité de la porosité au sein du sol. Les grandes valeurs indiquent un sol très transformé.

Lorsque l'on fait le lien entre modification du sol et dispersion, on s'aperçoit que la dispersion diminue clairement avec le degré de transformation, jusqu'à devenir nulle, et ne présente pas d'augmentation au delà d'un certain seuil de transformation. Tout se passe donc comme si les vers ne faisaient qu'améliorer physiquement leur milieu, dans la gamme de transformation étudiée. Nous n'observons donc pas de phase de dégradation de la niche. Deux explications sont possibles : soit cette dégradation n'a pas été observée car la durée de l'expérience était trop courte et le sol aurait pu être encore plus transformé par les vers, soit les vers ont transformé le sol jusqu'à un certain seuil optimal, à partir duquel ils ne font plus de transformation du sol mais seulement un entretien des structures produites (réseau de galeries).

2.2 Déterminants de la vitesse de dispersion

Nous avons vu que la tendance à disperser était influencée par un certain nombre de facteurs. Un résultat important est que la pré utilisation du milieu diminue la tendance à disperser. On peut dès lors se demander quel serait l'effet de l'utilisation du milieu de dispersion sur la vitesse de dispersion pendant la phase 2 de la dispersion (une fois que le patch de départ est quitté).

Il est difficile de répondre à cette question car il est difficile d'observer les vers de terre dans le sol. Jusqu'à récemment il n'existait aucune mesure de vitesse de déplacement des vers dans le sol. Pour remédier à ce problème nous avons adapté une technique basée sur les rayons X pour filmer le déplacement de vers de terre marqués dans le sol. Ce développement méthodologique a été réalisé en collaboration avec Anick Abourachid du MNHN.

L'expérience a consisté à filmer le passage successif de vers de terre au sein d'un dispositif de dispersion. La partie filmée, plus étroite, reliait deux compartiments plus larges. Les vers étaient introduits dans un seul des compartiments (Figure 32, Annexe 10).

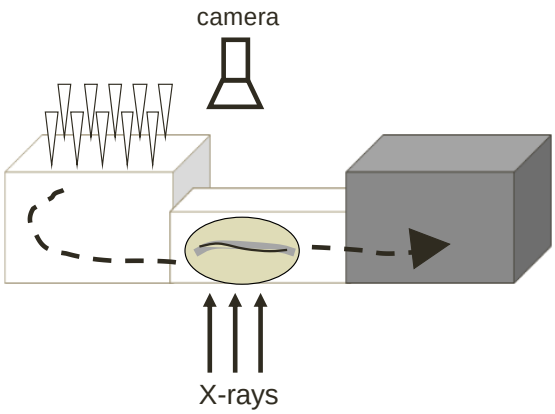


Figure 32 Schéma du dispositif pour filmer le mouvement des vers dans le sol. Les vers sont introduits dans la partie gauche et migrent vers la droite.

Les vitesses de déplacement des vers dans le sol ont ainsi pu être mesurées pour la première fois (Figure 33). Lorsqu'un vers passe dans un sol vierge de galeries, la vitesse est de l'ordre de 1 $cm.min^{-1}$ (60 $cm.h^{-1}$). Lorsqu'une galerie est présente, le vers l'emprunte et sa vitesse double (de l'ordre de 2 $cm.min^{-1}$). Il tire

bénéficie de la construction de la galerie par son prédécesseur. Lorsqu'un troisième vers traverse le sol, il empreinte la même galerie que les deux précédents, et sa vitesse est encore plus rapide, entre 2 et 8 $cm.min^{-1}$. Cette augmentation ne s'explique pas par l'aménagement du milieu car elle a été faite principalement lors du passage du premier ver. Ceci suggère que le troisième vers perçoit le passage des deux vers antécédents, et adopte une vitesse plus élevée. Il y aurait donc un mécanisme de reconnaissance des congénères.

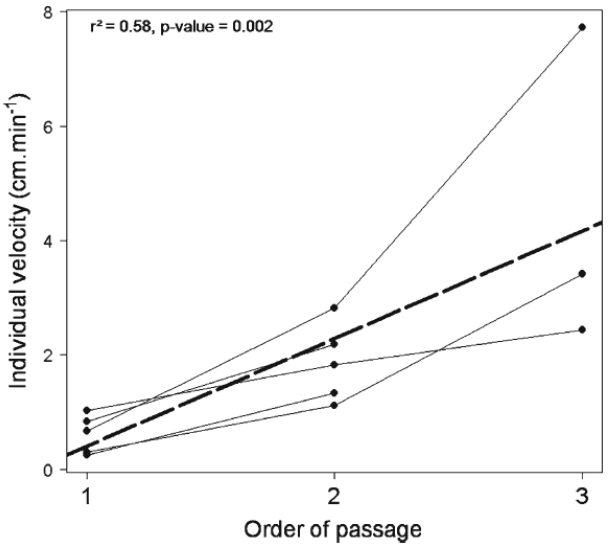


FIGURE 33 Vitesse de passage des vers lors des passages successifs dans la section de dispersion.

Cette expérience montre donc que les vers facilitent la dispersion de leur congénère par la construction de galeries, et suggère qu'il y a un mécanisme de reconnaissance du passage du passage de congénères dans les galeries.

3. PERSPECTIVES

L'étude des mécanismes de dispersion chez les vers a mis en évidence le rôle complexe et inattendu, en regard des théories existantes, des activités d'ingénierie des écosystèmes sur la dispersion. Nous avons vu que la transformation du milieu modifie de manière opposée l'aptitude à disperser selon le stade de la dispersion pendant lequel elle intervient, et selon son intensité. Ceci soulève une question générale qui est **le déterminisme des activités d'ingénierie chez les ingénieurs de l'écosystème**. Chez les organismes consommateurs, le niveau d'activité a été très bien étudié et modélisé, que ce soit chez les producteurs primaires, les herbivores ou les carnivores, en particulier avec les courbes de réponse fonctionnelle. En revanche il n'y a pas de cadre théorique prédisant le niveau d'activité des ingénieurs, sur des pas de temps écologiques ou évolutifs. La théorie de la construction de la niche est le cadre s'en rapprochant le plus. Cependant elle ne fait pas de prédiction quantitative, et reste peu étayée par des résultats empiriques.

Dans les années à venir, je propose de développer une vision quantitative des déterminants écologiques et évolutifs des activités des ingénieurs de l'écosystème. Les grandes étapes de cette démarche seront de quantifier la variabilité intraspécifique des activités d'ingénieur, puis d'identifier les déterminants écologiques et évolutifs de ces activités. Une partie importante sera dédiée aux aspects d'héritabilité au sens large, c'est-à-dire génétique, mais aussi écologique, qui risquent d'être particulièrement importants chez des organismes dont une partie de l'énergie est dédiée à la transformation du milieu.

Ce projet revêt de nombreuses implications appliquées, et sera développé dans le cadre de l'urbanisation, qui est une problématique majeure actuelle des changements globaux. Le monde s'urbanise à un rythme accéléré. Plus de 50% de la population mondiale vit aujourd'hui dans les zones urbaines. En raison de leur développement rapide, les zones urbaines sont susceptibles de jouer un rôle croissant dans les cycles biochimiques et sur la dynamique de la biodiversité mondiale. Le budget écologique des zones urbaines dépend fortement d'un certain nombre de services écosystémiques fournis par les organismes vivants - en particulier les ingénieurs de l'écosystème. Saisir dans quelle mesure les zones urbaines peuvent accentuer ou atténuer les conséquences des changements globaux nécessite de comprendre comment elles peuvent contraindre – sur des échelles écologiques mais aussi évolutives- l'activité des organismes biologiques clefs impliqués dans les cycles bio-géochimiques. Comprendre comment les zones urbaines affectent les ingénieurs de l'écosystème est donc crucial pour le développement durable des zones urbaines.

Le principal objectif de ce projet est d'étudier comment les environnements urbains peuvent déterminer l'écologie et l'évolution de certains traits et comportements d'ingénierie écologique. Les zones urbaines représentent de «nouveaux écosystèmes" pour les ingénieurs de l'écosystème car ils présentent des contraintes sociales et bio-physiques inédites. Cette nouveauté peut avoir déclenché des dynamiques éco évolutives inattendues, qui se doivent d'être identifiées. Les organismes n'ont été exposés que pendant une courte période de temps à ces nouvelles contraintes et ils sont à l'heure actuelle dans des processus d'ajustements écologiques et évolutifs. Les zones urbaines offrent ainsi une occasion unique - que nous ne devrions pas manquer - pour étudier en temps réel comment les organismes s'adaptent, à la fois écologiquement et évolutivement, à de nouveaux environnements.

Les deux étapes globales de notre perspective de recherche sont décrites brièvement :

1 Quelles sont les sources de variabilité dans les activités d'ingénierie de l'écosystème?

Déterminer les sources de variations des activités des ingénieurs de l'écosystème est nécessaire afin de prévoir leur activité dans un contexte précis. Les variations des traits peuvent être stochastiques (Fox & Kendall, 2002), induites par l'environnement, ou génétiques (West-Eberhard, 2003). Les variations stochastiques ne peuvent

pas être prédites. Le travail se concentrera donc sur les déterminants environnementaux et génétiques des activités des ingénieurs de l'écosystème.

a) Les facteurs environnementaux

Sur la base de corrélations faites sur le terrain, nous identifierons les caractéristiques environnementales clés qui influencent potentiellement les activités les ingénieurs de l'écosystème. Une fois identifiées, les normes de réaction à ces facteurs seront étudiées. Cette approche consiste à exposer les individus à des niveaux contrastés d'un facteur afin d'exprimer mathématiquement leur réponse à ce facteur. En comparant les normes de réaction aux génotypes, nous pouvons avoir une première idée de l'interaction entre le génotype et l'environnement.

b) composantes héréditaires de l'activité des ingénieurs des écosystèmes

Le niveau d'activité des ingénieurs de l'écosystème d'un individu peut être hérité des parents de deux manières: par l'héritabilité génétique traditionnelle (G), et par les effets parentaux à travers des modifications pré natales de l'environnement par les parents (effet de phénotype étendu) (E). Aucun de ces types d'héritabilité n'a encore été évalué chez les ingénieurs de l'écosystème. Ceci est cependant crucial car de nombreux sols urbains sont artificiels - sans modifications pré natales de l'environnement par les parents – ce qui peut avoir entravé l'héritabilité des activités d'ingénierie de l'écosystème chez les ingénieurs nouveau-nés.

Démêler ces deux sources d'héritabilité nécessite des plans expérimentaux spécifiques impliquant des élevages croisés (cross fostering), où les frères et sœurs sont échangés entre les environnements parentaux, afin d'éliminer l'effet de l'environnement. Ce type d'expérience sera réalisé en laboratoire et permettra d'estimer l'héritabilité h^2 en utilisant des régressions parent -enfants.

2 Valeur adaptative des activités ingénieurs en milieu urbain

Dans ce volet nous explorerons les relations entre le niveau d'activité des ingénieurs de l'écosystème et leur fitness dans différentes conditions environnementales, un point qui est crucial pour construire des modèles éco-évolutifs.

Cela nous permettra de déterminer la valeur adaptative de l'ingénierie, et en particulier de déterminer si les activités d'ingénierie sont corrélés à la fitness, ou au contraire, sont impliqués dans des compromis avec la capacité de reproduction par exemple. Cela se fera en laboratoire en soumettant des individus contrastés à différents environnements expérimentaux, avec la prédiction que la fitness dépendra de l'interaction entre gènes et environnement.

Globalement, cette perspective sera innovante concernant plusieurs aspects :

Tout d'abord, en évaluant le rôle des organismes sur l'infiltration de l'eau et l'incorporation de la litière dans les zones urbaines, elle développera l'écologie fonctionnelle et évolutive dans le domaine de l'écologie urbaine. Deuxièmement, en étudiant les sources de variabilité intra-spécifique et son héritabilité des comportements d'ingénierie de l'écosystème et des traits fonctionnels associés, elle contribuera à développer l'écologie évolutive dans le domaine de l'écologie des sols. Par conséquent, ce projet sera l'occasion de revoir en profondeur l'écologie des ingénieurs de l'écosystème, les approches d'ingénierie écologique, et le rôle des zones urbaines sur l'activité des organismes dans le contexte des changements globaux, à la fois à l'échelle écologique et évolutive.

Plus précisément, elle permettra 1) de déterminer si les variations spécifiques de traits intraspécifiques valent la peine d'être prises en compte dans les questions impliquant des ingénieurs de l'écosystème, en particulier dans les zones urbaines, 2) d'estimer dans quelle mesure les activités d'ingénierie de l'écosystème sont

héréditaires, 3) de déterminer comment les zones urbaines agissent comme filtre de la biodiversité génétique et phénotypique 4) d'apporter un cadre pour le développement des futures techniques d'ingénierie écologique dans les zones urbaines. Toutes ces questions n'ont pas encore reçu beaucoup d'attention, et ce projet contribuera à apporter des éléments de réponse appliqués et théoriques.

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ANNEXES

Annexe 1

Mathieu J., Davies J.T, Glaciation as an historical filter of below-ground biodiversity, Journal of Biogeography, 2014, 41:1204-1214

Journal of Biogeography (J. Biogeogr.) (2014) 41, 1204–1214

ORIGINAL ARTICLE

Glaciation as an historical filter of below-ground biodiversity

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ABSTRACT

Aim The latitudinal gradient in species richness is one of the most studied biodiversity patterns. Here we explore a north–south gradient in earthworm diversity, and evaluate the importance of current and historical filters in shaping the distribution of present-day below-ground species richness.

Location France.

Methods Using high resolution data on earthworm distributions across France, we document the latitudinal gradients in alpha (α), beta (β) and gamma (γ) diversity. We relate these gradients to species' traits, taxonomic aggregation and co-occurrence patterns, and correlate them with the present climate and the history of glaciation in Europe.

Results We found that γ-diversity decreases from south to north whereas α-diversity increases along the same latitudinal gradient. Communities in formerly glaciated regions are composed of smaller, more mobile species and show trait and taxonomical aggregation. In more southerly populations, which did not experience glaciation, earthworm species are larger, have smaller geographical ranges, and communities demonstrate a decrease in species co-occurrence resulting in lower local species richness.

Main conclusions We show that species richness gradients can present different – sometimes opposite – latitudinal trends depending upon the scale of the analysis. This scale dependence sheds new light on the underlying causes of global biodiversity gradients. The opposing latitudinal trends of the different components of diversity suggest that recolonization following glaciations during the Pleistocene acted as an environmental filter, and that competitive exclusion may be a more dominant ecological force in these former refugial areas. Overall our results show that past climate changes have left a deep footprint on present-day earthworm diversity patterns, from community to macroecological scales, and that different mechanisms of earthworm community assembly may predominate at different latitudes.

Keywords
Body size, community assembly rules, dispersal, earthworms, latitudinal gradient, past climate, range size, soil biodiversity.

INTRODUCTION

At global scales species richness of most major clades peaks in the tropics. The search for mechanistic explanations has typically focused on correlates with the present environment (Currie, 1991; Francis & Currie, 2003; Hawkins & Porter, 2003a; Buckley & Jetz, 2007; Powney *et al.*, 2010), calling upon processes such as latitudinal variation in evolutionary rates (Rohde, 1996; Allen *et al.*, 2002; Mittelbach *et al.*, 2007), or ecological explanations, including competition or environmental carrying capacity (see Willig *et al.*, 2003). However, mounting evidence suggests that historical processes are also important in structuring biodiversity gradients (Jansson & Dynesius, 2002; Hawkins & Porter, 2003b; Davies

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et al., 2011). Because contemporary environmental variables and historical process, such as the intensity of long-term climate oscillations, covary closely, distinguishing between drivers has proven difficult (e.g. Jansson & Davies, 2008). However, different processes should leave different signatures on the various components of diversity, namely alpha (α), beta (β) and gamma (γ). For example, rapid *in situ* speciation is predicted to result in high α-diversity plus high β-diversity and/or γ-diversity, whereas, species persistence through adaptive range shifts or migrations might result in more broad-ranged species, maintaining high α-diversity but low β- and/or γ-diversity.

To date, most analyses exploring regional diversity gradients have used range-map data, representing interpolated species distributions from sparse point location data, thereby limiting comparison between the various components of diversity. Recent evidence suggests that environmental correlates of species richness might vary with data type, for example point location versus gridded datasets (Hurlbert & Jetz, 2007). We suggest that these interpolated data might best represent γ-diversity, whilst point location captures α-diversity, perhaps leading to differences in environment–richness relationships between these different components of diversity. Few studies have explored variations in the strength of latitudinal gradients between differing diversity components because of the scarcity of suitable data at both broad and fine spatial scales (Meynard *et al.*, 2011; but see a recent paper by Kraft *et al.*, 2011).

Here, we decompose the latitudinal gradient for earthworm diversity into its constituent components (α-, β- and γ-diversity). We use a unique point dataset comprising a complete quantitative inventory of all earthworm species in France, representing over 1300 sites evenly distributed across the country (Fig. 1). In addition to its broad spatial extent and high resolution, this dataset is remarkable in its homogeneity in sampling quality as it was compiled in its entirety by M. Bouché (Bouché, 1972) using standardized protocols and taxonomy. Here, we describe the gradient in contemporary earthworm species richness, and relate it to climatic trends over the past 20,000 years.

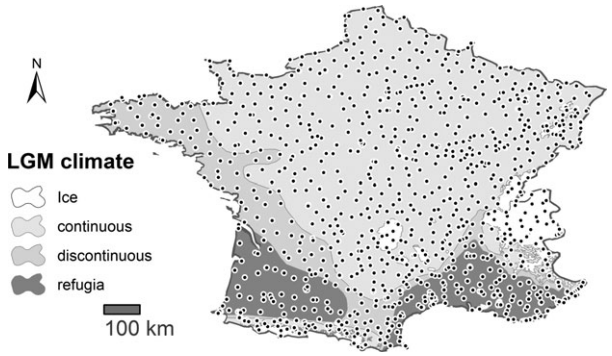

Figure 1 Sample sites of earthworms in France and permafrost limits during the Last Glacial Maximum (LGM, *c.*16 ka; according to Ehlers *et al.*, 2011). Ice, areas continuously frozen and under ice during the LGM; continuous, areas where the soil was continuously frozen during the LGM; discontinuous, areas where the soil was periodically frozen during the LGM; and refugia, areas that were not glaciated during the LGM.

The glacial history of France is well documented (e.g. Dercourt *et al.*, 2000; Buoncristiani & Campy, 2011; Calvet *et al.*, 2011) and the life history of earthworms suggests that current species distributions in previously glaciated regions must be explained by recent (post-glacial) migration and recolonization (Bouché, 1983). Our results therefore provide an example of a biodiversity gradient where historical processes are thought to be important, and we suggest that a better understanding of species' responses to historical climate change might help in predicting future responses, complementing autecology approaches such as niche-based distribution modelling. Understanding earthworm biodiversity in the context of climate changes is important because they play a prominent role in soil functioning and in the maintenance of ecosystems services (Bouché, 1977; Lavelle, 1988), and, at larger spatial scales, may be a major contributor to global carbon sequestration (Bossuyt *et al.*, 2005).

Based on our understanding of earthworm ecology and the history of climate change in Europe, we make the following predictions.

1. Earthworms will show a traditional latitudinal gradient with fewer species in the north.

2. Species currently at higher northern latitudes should be good dispersers and hence at these latitudes β-diversity will be low and range sizes large.

3. Species with northern distributions should be a subset of species found at more southerly latitudes as they have been through an historical filter of glacial history and, as a consequence, earthworm communities in northern latitudes should be more aggregated in their functional traits related to dispersion and range size.

4. Biotic processes, such as competition, should be more important in structuring communities in the south where there has been a longer history of continuous coexistence.

MATERIALS AND METHODS

Data sets

Species distribution data were obtained from Bouché (1972), and comprise the abundance of all earthworm species in France across more than 1300 sites (Fig. 1), using a standardized sampling protocol. Each site represents an area of homogenous land use, typically covering 1 ha or more. Sites were spaced up to 30 km apart (Fig. 1), and sampling was performed between 1963 and 1968. Each region was sampled in at least two different years. Samples were taken during autumn and spring, the best climatic periods for earthworms. The basic environmental features of all sites, including location, vegetation cover, elevation, soil chemical properties are reported in Bouché (1972). Data are available from the Dryad Digital Repository (see Data Accessibility). Earthworm diversity was sampled by collecting three blocks of soil

(1 m × 1 m × 0.3 m) at each site, which were sorted with a wet sieving machine designed specifically for this task. Juveniles, which cannot be identified accurately to species, were reared until maturity to allow identification. We complemented these data by compiling a database of the range maps of French species across Europe, based on the Fauna Europaea database (de Jong, 2013).

For the set of 105 species present in France, we synonymized taxonomy and species names according to the classification of Fauna Europaea (de Jong, 2013) and compiled information on more than 30 anatomical traits, based on Bouché (1972) and Sims & Gerard (1985). We selected traits related to mobility, ecological preferences and reproduction because we a priori expected such traits to be subject to filtering by environment or involved in competition (see Appendix S1 in Supporting Information for the full list of traits). Finally, we obtained data on the limits of permafrost and refugia during the Late Glacial Maximum (LGM) from several sources (Dercourt *et al.*, 2000; Buoncristiani & Campy, 2011; Calvet *et al.*, 2011).

Climatic data for the present and the LGM were extracted from the ECHAM3 palaeoclimatic model (Braconnot *et al.*, 2007) for mean annual temperature (MAT) and mean annual precipitation (MAP). Habitat complexity was characterized as the variance in elevation ($Elev_{var}$), measured as the standard deviation in elevation within each region. Elevation data were retrieved from the French National Institute of Geography, at a resolution of 25 m (Bd alti database, http://www.ign.fr/).

Biodiversity gradient analysis

To explore the latitudinal gradient in earthworm biodiversity, we compared α-, β- and γ-diversity components. Alpha diversity is the local species richness at each site. Gamma diversity represents the regional species pool, defined as the 150-km radius around each site: a reasonable scale given the geographical distribution of plots and the relatively limited dispersal distance of earthworms (Eijsackers, 2011). To correct for unequal sampling within regions, we used bootstrapping ($n = 100$ replicates) to randomly select the same number of sites ($n = 28$ sites) per region, and computed diversity across this subset. Beta diversity (β_{SOR}) was estimated within regions following the approach of Balsega and colleagues (Baselga, 2010), and was decomposed into a spatial turnover component (β_{SIM}) and a nestedness component (β_{NES}). The relationship between explanatory variables and the different components of diversity was analysed following Baselga (Baselga, 2012), using Pearson's correlations (r) and Dutilleul's correction for the presence of spatial autocorrelation (Dutilleul, 1993).

Species geographical range size

We used the observed distribution of species in Europe as an indicator of species geographical range size. First, the geographical range of each species was calculated by summing the area of the countries in which the species occur, based on distribution maps of Fauna Europaea and the published literature. Second, we determined the average community geographical range size at each sampling point, by computing the average range size of the species present at each location.

Cumulative species plot

We used cumulative species plots to explore the role of past glaciations as filters on present-day communities. First, we calculated the accumulation of species diversity in 1.2° latitudinal bands moving from south to north. Second, we repeated the procedure, but moving from north to south. If, as we predict, northern species are a subset of species in the south, only a few additional species will be recorded moving from south to north, and the cumulative species plot should initially be steep then shallow or flat. In contrast, there should be an initially much shallower cumulative plot moving from north to south. The further south the intersection between the two curves, the greater the evidence suggesting that the more northern species pool is a subset of the southern pool. In our analysis, the two most southerly bands represent mainly non-glaciated areas (NG), while the more northerly bands were either under discontinuous or continuous permafrost during the LGM. Variation in sampling effort per band (i.e. number of sites) was corrected by bootstrapping with maximal equal sampling size per band.

Community evolutionary structure

In the absence of a well-resolved phylogeny for earthworms, we characterized the evolutionary structure of communities across and within sites using the species-to-genus ratio by calculating the mean number of species per genus for each site with more than one species (Simberloff, 1970). Overall, 105 earthworm species are present in France, distributed across 29 genera and six families. Sites with many species per genus might be considered to be taxonomically or evolutionarily aggregated, whereas sites of equivalent richness but with few species per genus might be considered to be taxonomically or evolutionarily dispersed. In order to test the degree of taxonomical aggregation we used a null model approach which compared the observed ratio of species per genus with expectations from randomly assembled communities with the same species richness.

In addition, we quantified the variation in latitudinal range at the species level that can be explained at different taxonomic levels (see Hof *et al.*, 2010). We performed variance component analyses (VCA) and analysis of similarity (ANOSIM), with a restricted maximum likelihood approach, to test the significance of the observed pattern using the functions 'lme' and 'varcomp' in the APE package within R (Paradis, 2012). A large proportion of the species variance explained at higher taxonomic levels would indicate strong phylogenetic structure in latitudinal range.

We evaluated the functional diversity among sites by constructing a distance tree using 32 species characteristics (see Appendix S1). These characteristics included morphological and anatomical traits that are commonly used to identify species, and which are related to functional aspects of earthworm ecology. Anatomical traits mainly included measures concerning the presence and the position of particular organs: it is of interest to note that such traits are believed to have evolved in relation to burrowing and feeding activity, key functional aspects of earthworm ecology (Sims & Gerard, 1985). We used a hierarchical ascendant classification (CAH) with Euclidean distance and the Ward algorithm to cluster species. Usefully, branch lengths on the tree represent morphological distances, and can be used to calculate functional divergence between species and trait aggregation within communities (Petchey & Gaston, 2002).

Competition versus filtering

We explored the functional structure of communities across sites using the distance tree and metrics developed within the ecophylogenetics literature that compare the mean pairwise distance (MPD) among taxa between sites, more typically used to describe the phylogenetic clustering of species (Webb *et al.*, 2002). Low MPD suggests under-dispersion (species are more similar), whereas high MPD suggests over-dispersion (species are less similar). We compare the empirical distributions of MPD to a null model generated from randomly shuffling species membership across sites whilst keeping site species richness constant. Over-dispersion is traditionally thought to indicate evidence for competition (similar species displace each other), whereas under-dispersion is thought to reflect filtering processes (Webb *et al.*, 2002). If, as we predicted, competition is more important in structuring communities in the south, these communities should show a greater tendency towards over-dispersion, whilst communities in the north should be more aggregated because of the historical filter of past glaciations.

Quantification of species co-occurrence patterns

We used the *C*-score (Gotelli, 2000), a quantitative index of species co-occurrence, as an indicator of the strength of competition in communities. The *C*-score is defined as $(R_i - S) \times (R_j - S)$ where R_i and R_j represent the total number of occurrences of species i and j, respectively, and S is the number of shared occurrences. The average *C*-score, calculated over all unique species pairs, summarizes the pattern of co-occurrence as a single metric. Significance was assessed by constructing random communities ($n = 200$) at each site, shuffling the species present in the regional pool using the same regions as for the latitudinal diversity gradient analysis above. This approach requires fixing both the number of species by site and the number of occurrence of each species, which is a good compromise between Type I and Type II errors (Gotelli, 2000; Gotelli & Entsminger, 2003). We then compared the value of the observed *C*-score with the distribution of null *C*-scores to estimate the probability of non-random species co-occurrence.

Geographical representation

As illustration, we generated maps of average community species range, number of species per genus, maximum body size in the community, mean trait dispersion and deviance in *C*-scores from the null model, interpolating values by punctuated kriging using the cross-validated semivariograms and a weighted linear combination of 15 surrounding data points. Statistical tests were only performed on sampled points.

RESULTS

Latitudinal diversity gradients

Regional earthworm species richness (γ-diversity; Fig. 2a) decreases with latitude ($r = -0.59$, $P = 0.05$, Table 1) and increases with LGM temperature ($r = 0.56$, $P = 0.05$) and present precipitation ($r = 0.35$, $P = 0.03$). Beta diversity, quantified by Sørensen's index, follows trends for γ-diversity ($r = -0.75$, $P = 0.03$), with higher turnover at lower latitudes (Fig. 2b), and positive correlation with LGM temperature ($r = 0.77$, $P = 0.01$) and present precipitation ($r = 0.47$, $P = 0.01$). However, decomposing β-diversity into its separate components (Table 1, Appendix S2) reveals that turnover and nestedess demonstrate different trends with

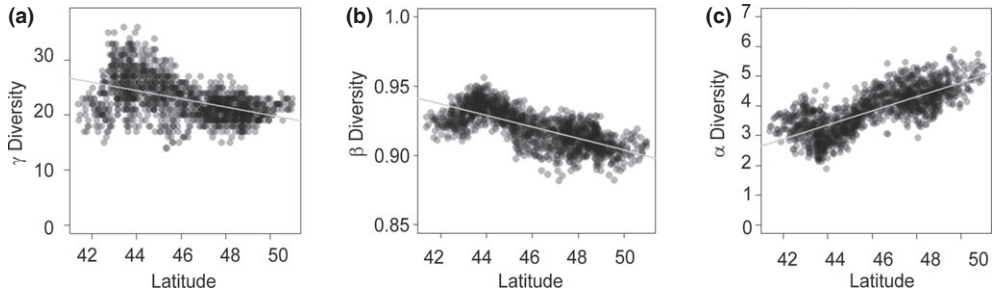

Figure 2 Decomposition of the earthworm latitudinal diversity gradient within France into (a) gamma (γ), (b) beta (β), and (c) alpha (α) diversity.

Table 1 Values for Pearson's correlation coefficients (r) between each explanatory variable and the different features of earthworm communities in France.

	γ-diversity	β_{SOR}	β_{SIM}	β_{NES}	α-diversity	Species range	species/genus	Body size	Traits dispersion	d-*C*-score
Latitude	**−0.59 (0.05)**	**−0.75 (0.03)**	**−0.57 (< 0.01)**	**0.31 (< 0.01)**	**0.77 (0.02)**	**0.39 (0.05)**	**0.38 (0.04)**	**−0.26 (< 0.01)***	**−0.14 (0.05)**	**−0.45 (0.05)**
$Elev_{var}$	0.15 (0.51)	0.21 (0.39)	0.07 (0.12)	**0.09 (< 0.01)**	−0.37 (0.10)	−0.12 (0.40)	−0.19 (0.15)	0.13 (0.26)	0.02 (0.73)	0.08 (0.58)
MAP_{LGM}	0.19 (0.37)	0.17 (0.43)	0.07 (0.12)	0.04 (0.19)	−0.24 (0.27)	−0.02 (0.88)	−0.12 (0.24)	0.16 (0.12)	−0.05 (0.34)	0.07 (0.64)
MAT_{LGM}	**0.56 (0.05)**	**0.77 (0.01)**	**0.65 (< 0.01)**	**−0.42 (< 0.01)**	**−0.70 (0.01)**	**−0.43 (0.02)**	**−0.34 (0.01)**	0.26 (0.08)	**0.19 (< 0.01)**	**0.48 (0.02)**
MAP	**0.35 (0.03)**	**0.47 (0.01)**	**−0.42 (< 0.01)**	**0.30 (< 0.01)**	−0.34 (0.40)	**−0.34 (< 0.01)**	**0.08 (0.01)**	**0.22 (0.01)**	**0.21 (< 0.01)**	**0.25 (0.05)**
MAT	−0.2 (0.91)	−0.01 (0.97)	−0.05 (0.17)	**0.08 (0.02)**	−0.08 (0.60)	0.07 (0.45)	**0.07 (0.04)**	0.04 (0.59)	**−0.1 (0.03)**	−0.01 (0.97)

β_{SOR}, intra-regional beta diversity (following Baselga, 2010); β_{SIM}, spatial turnover; β_{NES}, nestedness; d-*C*-score, difference between observed and simulated *C*-score in the null model; $Elev_{var}$, variance in elevation; MAP_{LGM}, mean annual precipitation for the Last Glacial Maximum (LGM) period; MAT_{LGM}; mean annual temperature for the LGM period; MAP, current annual precipitation; MAT, mean current annual temperature. Bold values are statistically significant ($P < 0.05$). The associated probabilities following Dutilleul's (1993) correction for the presence of spatial autocorrelation are shown in brackets. * Body size is significantly related to latitude^2 but not to latitude ($r = -0.27$, $P = 0.11$).

environment. Turnover decreases with latitude ($r = -0.57$, $P < 0.01$) and present precipitation ($r = -0.42$, $P < 0.01$) but increases with LGM temperature ($r = 0.65$, $P < 0.01$). In contrast, nestedness increases with latitude ($r = 0.31$, $P < 0.01$), present precipitiation ($r = 0.3$, $P < 0.01$) and variance in elevation ($r = 0.09$, $P < 0.01$), but decreases with LGM temperature ($r = -0.42$, $P < 0.01$). Overall, at the regional scale, earthworm species richness is higher, turnover in species composition is greater, and communities are less nested at lower latitudes and where temperatures at the LGM were warmer. However, local species richness (α-diversity; Fig. 2c) shows an unexpected and rarely reported countergradient, with diversity increasing towards higher latitudes ($r = 0.77$, $P = 0.02$) and decreasing with LGM temperature ($r = -0.70$, $P = 0.01$).

Most endemic and narrowly distributed species are found in the south (Appendix S3), while species at higher latitudes have greater latitudinal extents (Fig. 3a, $r = 0.39$, $P = 0.05$, from the correlation of range size against latitude, Table 1). In addition, species range is negatively correlated with LGM temperature ($r = -0.43$, $P = 0.02$) and present precipitation ($r = -0.34$, $P < 0.01$). Interestingly, species range is conserved at the genus level (VCA: 38% of variance, ANOSIM: $r = 0.29$, $P < 0.01$, Fig. 3b), but not at the family or order level (VCA = 1 and 14%, ANOSIM: $r = 0.004$, $P = 0.45$ and $r = 0.43$, $P = 0.14$ for family and order, respectively).

Community assemblage patterns

Moving from south to north we find that the cumulative species plot is initially steep, and converges on an asymptote at approximately 46º N (Fig. 4a), indicating that many new species are encountered as we cross southern latitudinal bands while few new species are included as we approach more northerly latitudinal bands. In contrast, the cumulative species plots moving from north to south is initially flat but steepens significantly when reaching non-glaciated latitudes (at approximately 46º N), as additional species not present in the northern sites are picked up (Fig. 4a). The two curves intersect at around 44º N, suggesting that species-rich northern latitude communities above 44–46º N are composed of a subset of species from more southerly latitudes. By comparing species in the south (below 44° N) to species in the north (above 46° N), controlling for sampling effort, we find that 62% of total species are found only in the south while only 10% are found only in the north. Overall, only 28% of species are present in both the south and the north. Three further lines of evidence provide additional support for this nested relationship. First, although the total number of genera in the north is less than that found in the south, the number of species per genus (taxonomic aggregation) increases towards the north (Fig. 4b,c, Table 1). Second, body size shows a significant latitudinal gradient (Fig. 5a, Table 1, $r = -0.26$, $P < 0.01$, from the regression of body size against latitude), with large-bodied species concentrated in the south [below 44° N: average = 28 cm and

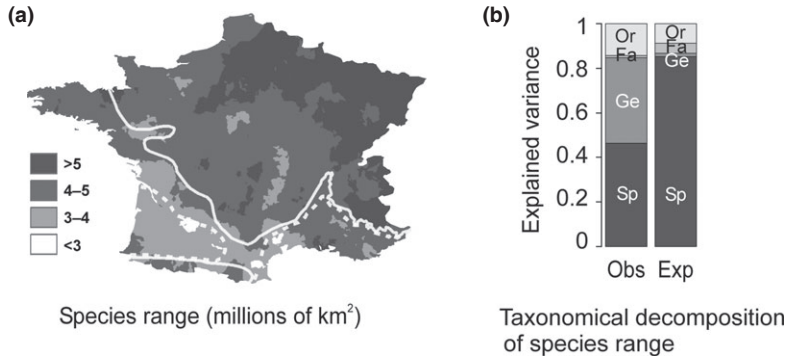

Figure 3 Distribution of earthworm species range sizes across France. (a) Geographical range size, calculated as the mean range extent in Europe, based on Fauna Europaea, and mapped across current earthworm distributions in France. (b) Decomposition of earthworm species' range size variance (VCA) according to taxonomy. Sp = Species, Ge = Genus, Fa = Family, Or = Order. Variance was scaled to one. Mapped variables were partitioned into four classes.

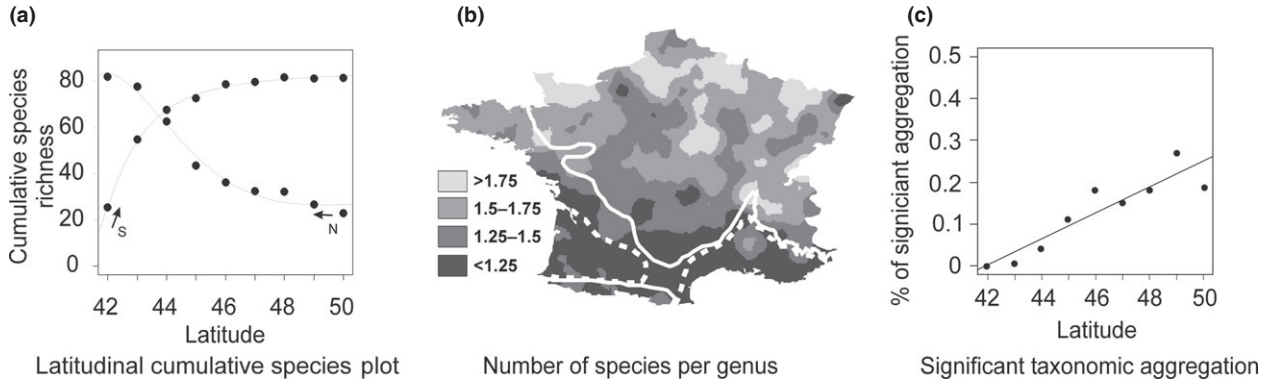

Figure 4 (a) Cumulative number of earthworm species moving from north to south (N) and from south to north (S) in France. (b) Distribution of taxonomic aggregation. The solid white line indicates the limits of continuous permafrost during the Last Glacial Maximum (LGM). Dashed lines indicate the limits of discontinuous permafrost during the LGM. (c) Percentage of significant taxonomic aggregation across latitude, tested with a null model drawing random communities with the same species richness. Mapped variables were partitioned into four classes.

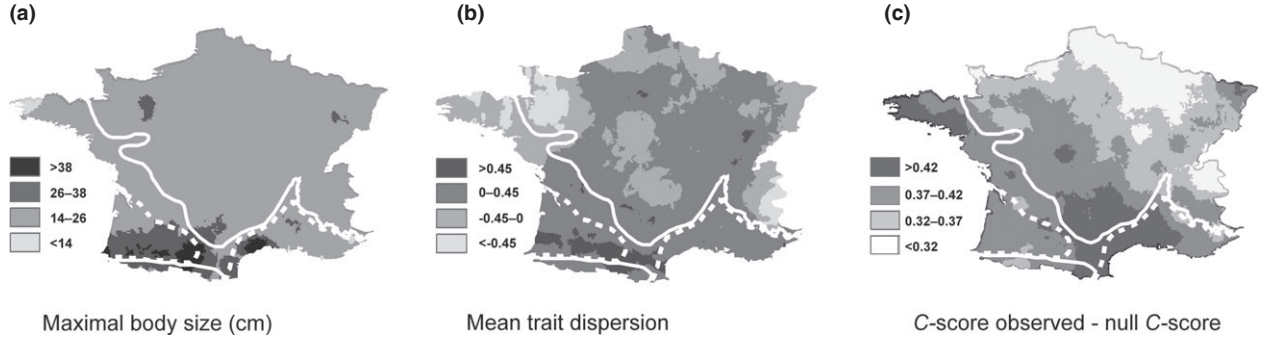

Figure 5 (a) Average maximal species body size per earthworm community mapped across multiple communities in France. (b) Map of trait aggregation. (c) Deviation between observed and null *C*-scores (see main text). Solid white lines indicate the limits of continuous permafrost during the Last Glacial Maximum. Dashed lines indicate the limits of discontinuous permafrost during the LGM in France**.** Mapped variables were partitioned into four classes.

max = 105 cm (several large species); while above 46° N: average = 20 cm and max = 57 cm (only 1 rare large species)], whereas small species (< 20 cm) are ubiquitous south to north. Three, trait dispersion – which is usually interpreted as an evidence of species filtering by environmental constraints – decreases from south to north (Fig. 5b,

Table 1, $r = -0.14$, $P = 0.05$, from the regression of standardized MPD against latitude), thus traits are more aggregated in the north than in the south of France. Further, the correlation with trait dispersion is even stronger for MAT ($r = 0.21$, $P < 0.01$) and MAT_{LGM} ($r = 0.19$, $P < 0.01$). We therefore suggest that earthworms in the north and where temperatures were colder represent a subset of more southerly species that have been filtered on species traits related to dispersal ability (i.e. body size) and temperature tolerances.

Finally, to evaluate evidence for competition, we used the *C*-score index of species co-occurrences, which quantifies the degree of species distribution overlap. The larger the *C*-score, the higher the proportion of potential species pairs found not to co-occur naturally and, by implication, the greater the role of competition in structuring community assembly. Earthworm *C*-scores increase strongly from north to south (Fig. 5c, Table 1, $r = -0.45$, $P = 0.05$, for the regression of *C*-score against latitude), and with temperatures at the LGM ($r = 0.48$, $P = 0.02$), suggesting that competition was more important in the south, where communities have been less exposed to climate change and therefore have a longer history of competitive interactions.

Environmental correlations

In general, we found that temperature at the LGM (MAT_{LGM}) was as good as or better than latitude in predicting earthworm diversity gradients (Table 1). In contrast, correlations with present-day temperature (MAT) and environmental heterogeneity ($Elev_{var}$), characterized as variance in elevation, were mostly non-significant. Although correlations with present-day precipitation patterns (MAP) were significant, with only a single exception ($r = 0.21$ and 0.19 for correlations of trait dispersion against MAP and MAT_{LGM}, respectively; Table 1), correlation strengths were always higher for MAT_{LGM}.

DISCUSSION

Traditional but also unexpected patterns of biodiversity

Regional trends in earthworm diversity match classical diversity gradients with higher diversity towards the tropics. A number of mechanisms have been proposed to explain this remarkably ubiquitous biogeographical trend (see Willig *et al.*, 2003 for a review). However, by comparing α, β and γ components of earthworm diversity we reveal an unusual reverse gradient, with higher α-diversity towards the pole. This reverse latitudinal gradient has been observed in only a few organisms (e.g. Crow, 1993; Bolton, 1994; Skillen *et al.*, 2000; Chown *et al.*, 2004), and has typically been explained by local-scale heterogeneity associated with geography, geology, hydrology, or history (Skillen *et al.*, 2000; Willig *et al.*, 2003). Our study illustrates a reversal in the latitudinal diversity gradient among the various components of diversity. Previous studies that have simultaneously analysed latitudinal variation in α-, β- and γ-diversity in other organisms (Kaufman, 1998; Clarke & Lidgard, 2000; Stevens & Willig, 2002) show a generally positive (but sometimes no) association between the three components of diversity. We suggest that past glaciations acting as an historical filter, in conjunction with present climate and topography, explain this unique diversity gradient for earthworms.

Dispersal ability as a filter of biodiversity

Earthworms are unable to persist in permafrost over long periods, such as experienced during the LGM (Holmstrup *et al.*, 1991). Even species that burrow in the ground to avoid frost (Nuutinen & Butt, 2009) were not able to do so during this period as the deep layers of soil were frozen. In consequence, it is usually accepted that glaciations during the LGM extirpated all earthworms species from northern latitudes (Tiunov *et al.*, 2006), hence species currently found at these latitudes must have recently recolonized from historical refugia. This hypothesis is consistent with previous interpretations of earthworm distribution, and with the distribution of various other Northern Hemisphere taxa (e.g. Bennett *et al.*, 1991; Hewitt, 1999; Hawkins & Porter, 2003b; Petit *et al.*, 2003; Habel *et al.*, 2005; Svenning & Skov, 2007).

Recolonization of northern France required a combination of good dispersal capacity and some degree of niche plasticity. Dispersal ability may therefore have acted as a filter on these species. Theory predicts that filtering results in reduced variability of species traits, referred to as trait aggregation (Keddy, 1992). As niches and traits are typically phylogenetically conserved, filtering can also result in taxonomic aggregation – an increase in the relative number of species per genus – because species in some genera (possessing beneficial traits) will be favoured over species within other genera (lacking such traits).

Although the phylogeny of earthworms is still not well resolved, and taxonomical issues such as cryptic species may introduce some noise in the data, our results provided four strong lines of evidence suggesting that earthworms were filtered into more northerly, previously glaciated, communities: (1) there is an increase in trait aggregation from south to north; (2) communities show taxonomic aggregation, with a higher ratio of species per genus in the north and many southern species falling into small genera not found within more northern communities; (3) species in northerly communities are a subset of species in more southerly communities; and (4) communities in the north are composed of species with wider geographical range size (which demonstrates taxonomic conservatism) than in the south, a pattern which has been reported widely in other taxa (Stevens, 1989; Rohde, 1996).

Further, we show that diversity gradients correlate most strongly with temperatures at the LGM, and that correlations with present-day temperatures or environmental heterogeneity were generally weaker or non-significant. Our results

indicate strongly that historical climate changes have left a deep footprint on present-day earthworm diversity via selective recolonizations following glacial retreats at the end of the LGM. Interestingly, our results correspond with a recent study on scarab bettles (Hortal *et al.*, 2011), a group that nest in the ground, and therefore are also sensitive to permafrost. Scarab beetles in the north were also found to be a nested subset of those in the south, and phylogenetically clustered.

Body size as a key trait

Identifying the traits that determine a species' ability to colonize or invade new habitats is a challenge. We note that only earthworm species that were able to recolonize the north of France and Europe are invasive in Canada and northern USA – regions that were otherwise devoid of indigenous earthworm fauna due to Pleistocene glaciations (Hendrix, 1995) – suggesting that they possess particular traits that predispose them to range expansion, such as high dispersal capacity. We show that these species are on average smaller than those species restricted to former refugia. In earthworms, body size, which ranges from 1.8 to 105 cm in France (Bouché, 1972), is strongly related to demographic parameters (Evans & Guild, 1948; Lavelle, 1981), an important determinant of species' colonizing capacities at large spatial scales. First, larger species produce larger cocoons deposited deeper in the ground (Lavelle, 1981), which are less likely to be transported accidentally by other animals or by humans (Marinissen, 1992). Second, body size also differentiates species with respect to their strategy of desiccation resistance during dry months: small species spend this period at the cocoon stage, while large species enter into diapause or quiescence, waiting for autumn to complete their life cycle, which increases their generation time (Bouché, 1977). Therefore, bigger species require more time to reach maturity and complete their life cycle, and are typically considered to be *K*-strategists, whilst small species are more *r*-strategists (Bouché, 1977; Satchell, 1980), which is associated with greater invasiveness (Ehrlich, 1984).

Interestingly, our results run contrary to that predicted by Bergmann's rule, which suggests that physiological constraints lead to larger body sizes in colder climates (e.g. at higher latitudes), although as originally formulated the rule applied to intraspecific variation in endotherms. As suggested by Shelomi (2012), Bergmann's rule should be applied with caution to ectothermic taxa.

Historical refugia: haunted by the ghost of competition past?

In former refugia, climatic conditions were more suitable for species persistence, explaining higher total regional diversity; however, we find that local (α) diversity is lower than observed across more northerly sites. In addition, community *C*-scores reveal a lower index of species co-occurrence, and higher trait dispersion in the south, although trends are relatively weak. These results are consistent with the signal of competition shaping community structure in historical refugia: competition theory predicts a lower rate of species co-occurrence and higher trait variability where competitive interactions are strong. We suggest that the much longer history of competitive interactions in former refugia may have resulted in greater competitive exclusion and a decrease in local species richness in the south. However, other factors, including isolation and diversity of glacial refugia, might have also contributed to observed richness patterns in this region. Further work is required to evaluate these hypotheses more fully; for example, more comprehensive phylogenetic information is required to evaluate patterns of co-occurrence among close relatives, and identify cryptic species that are not easily distinguished by morphology.

CONCLUSIONS

Earthworms across France demonstrate two ecological gradients that run counter to classic diversity patterns: α-diversity is higher at more northerly latitudes and body size decreases from south to north. By using data on environment at the LGM, we reveal how consideration of historical process can help in our understanding of present-day diversity patterns. We show that the imprint of glacial history is apparent statistically in gradients of species richness, species range distributions and the aggregation of species traits at both macroecological scales and at the community level. We suggest that past glaciations have acted as an historical climate filter on dispersal ability, resulting in opposing latitudinal gradients for the different components of earthworm diversity. Previously glaciated (more northerly) communities are composed of species with larger geographical ranges, are functionally and taxonomically aggregated, and show low spatial turnover in species composition (low β-diversity), despite high local species richness (α-diversity). This historical filtering process is also apparent in the lower regional (γ) diversity of northern communities, from which poor dispersers (species with larger body sizes) were filtered out. In the north, earthworm communities are assemblages of past invaders, while communities within former LGM refugia may have been structured more by competitive interactions, perhaps over much longer timeframes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 List of species' anatomical traits used in this study.
Appendix S2 Latitudinal decomposition of beta diversity into (a) β_{SIM} = turnover and (b) β_{NES} = nestedness, following Baselga (2010).
Appendix S3 Map of the number of endemic species.

DATA ACCESSIBILITY

All data mentioned in this study are available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.g7046 as two text files. The first file contains the community data set, with the presence/absence of all earthworm species at each location, and with a brief description of each location. The second file describes the traits of all earthworm species in France, according to Bouché (1972).

BIOSKETCHES

Jerome Mathieu is an assistant professor at the University Pierre & Marie Curie (UPMC), France. His research focuses on the factors driving the spatial distribution of soil biodiversity.

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Annexe 2

M. Torres-Leguizamon, J. Mathieu, T.Decaens, and L. Dupont, Genetic Structure of Earthworm Populations at a Regional Scale: Inferences from Mitochondrial and Microsatellite Molecular Markers in *Aporrectodea icterica* (Savigny 1826). PlosOne, 2014, e101597

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Genetic Structure of Earthworm Populations at a Regional Scale: Inferences from Mitochondrial and Microsatellite Molecular Markers in *Aporrectodea icterica* (Savigny 1826)

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Abstract

Despite the fundamental role that soil invertebrates (e.g. earthworms) play in soil ecosystems, the magnitude of their spatial genetic variation is still largely unknown and only a few studies have investigated the population genetic structure of these organisms. Here, we investigated the genetic structure of seven populations of a common endogeic earthworm (*Aporrectodea icterica*) sampled in northern France to explore how historical species range changes, microevolutionary processes and human activities interact in shaping genetic variation at a regional scale. Because combining markers with distinct modes of inheritance can provide extra, complementary information on gene flow, we compared the patterns of genetic structure revealed using nuclear (7 microsatellite loci) and mitochondrial markers (COI). Both types of markers indicated low genetic polymorphism compared to other earthworm species, a result that can be attributed to ancient bottlenecks, for instance due to species isolation in southern refugia during the ice ages with subsequent expansion toward northern Europe. Historical events can also be responsible for the existence of two divergent, but randomly interbreeding mitochondrial lineages within all study populations. In addition, the comparison of observed heterozygosity among microsatellite loci and heterozygosity expected under mutation-drift equilibrium suggested a recent decrease in effective size in some populations that could be due to contemporary events such as habitat fragmentation. The absence of relationship between geographic and genetic distances estimated from microsatellite allele frequency data also suggested that dispersal is haphazard and that human activities favour passive dispersal among geographically distant populations.

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Introduction

Earthworms represent one of the largest reservoirs of animal biomass and the main invertebrate group of soil ecosystem engineers in most terrestrial temperate ecosystems [1]. They play a key role in soil functioning: they relocate surface litter or organic matter throughout the soil profile [2,3], affect microbial activity [4], and have a significant effect on organic matter mineralisation and soil biogeochemical cycles [5]. They modify soil structure *via* the construction of biogenic aggregates and galleries [6], resulting in differences in aeration and drainage [7]. Earthworms also influence plant growth [8] and plant community structure [9,10], and can be used as indicators of habitat quality [11,12] and as biomarkers in toxicity tests [13].

Despite their fundamental impact on soil ecosystems, the spatial population dynamics of earthworms is poorly understood. In particular, there is little information on the amount and spatial distribution of genetic variation in earthworm populations. Few studies have simultaneously investigated the influence of historical events, such as glacial periods, and contemporary processes, such habitat fragmentation, on the genetic diversity of these species. In their review on the genetic structure of soil invertebrate populations, Costa et al. [14] cite only seven studies of earthworm populations. They conclude that earthworm populations generally show a complicated pattern of gene flow, with a weak relationship between genetic and geographic distances. Population genetic structure of earthworms is therefore likely to be strongly influenced by human activities. In an agricultural landscape, the spatial distribution of earthworm species is expected to be fragmented, with patches of suitable habitat being separated by large areas of unsuitable habitat. Furthermore, it has been shown that earthworms are negatively affected by the intensity of agriculture [15] and in particular by the use of pesticides [16]. The consequences of landscape spatial structure on genetic diversity depend on the rate at which individuals move between patches of suitable habitat. In particular, restricted dispersers, such as earthworms, are likely to be prone to local extinction due to stochastic processes [17]. However, it has also been suggested that the rate of gene flow and the amount of genetic variation may actually increase as habitats

become more fragmented [18]. More earthworm population genetics studies are needed to determine (i) how earthworms move between patches, (ii) how spatial structure affects the stability and dynamics of spatially structured earthworm populations, and (iii) how the landscape affects genetic diversity.

Our model earthworm species, *Aporrectodea icterica* is an abundant species commonly found in agricultural soils [19]. It belongs to the endogeic ecological type (i.e. species living and foraging in or immediately below the rhizosphere making horizontal burrows through the soil to move around and to feed) [20], although *A. icterica* is also believed to feed at least partly on leaf litter [21]. This diploid, obligatory bi-parental species [22] is native to the temperate zones of Europe [20], but is an invasive species in North America [23]. Its taxonomic status is firmly grounded and the species has distinct morphology making it easy to recognise. Its dispersal behaviour has been studied in laboratory [11,24] and it has been used in ecotoxicological studies [19,25,26]. At the genetic level, a recent study of two *A. icterica* populations revealed the existence of two mitochondrial lineages with divergence values ranging from 10% to 11% [27]. Such highly divergent mitochondrial lineages have been reported in several other earthworm morphospecies (e.g. [28,29,30]). In *A. icterica*, nuclear analysis indicates that the two lineages interbreed [27], demonstrating that they belong to the same species.

Deep mitochondrial divergences within morphospecies can be attributed to population isolation within distinct periglacial refugia [29]. When the divergent lineages were found in sympatry, such as in *A. icterica*, it was suggested that lineages came into contact and mixed during recolonisation, during the warmer interglacial periods [29]. Given the low vagility of earthworms, we hypothesise that this mixing is in large part due to human activities which have accelerated the rate of organism dispersal, and brought previously allopatric species into contact [31]. During this secondary contact, weak reproductive barriers between lineages and fertile hybrids with little or no reduction in fitness can lead to genetic assimilation and loss of genetic distinctness between the hybridizing lineages, and the possible extinction of one or both parental lineages [31]. For recent or in progress hybridization events, introgressed mitochondrial and nuclear genes are predicted to display cyto-nuclear disequilibrium [32] (i.e. non-random association of alleles or genotypes at a nuclear locus with haplotypes of cytoplasmically inherited organellar DNA [33]).

Here, we focus on the genetic structure of seven earthworm populations sampled at a regional scale (<100 km^2), comparing the spatial regional patterns obtained using mitochondrial (mtDNA) and nuclear (nDNA) molecular markers. We discuss the role of evolutionary forces including genetic drift and contemporary gene flow, large-scale landscape changes (e.g. glacial periods) and anthropogenic effects in structuring the genetic diversity and in the differentiation of populations.

Materials and Methods

Sampling and DNA extraction

In April 2010, 218 *A. icterica* individuals were collected from seven populations in Normandy (northern France). Six populations were located in >6 year-old pastures (on average, clay = 16%, silt = 64% and sand = 20%, mean pH: 6.1, C: 23 g.kg^{-1}, N: 2.3 g.kg^{-1}, C/N: 10), within a distance of 3 to 10 km from the city of Yvetot (I03, I07, I19, I20, I25 and I27). Each proprietor gave his agreement for sampling to J. Mathieu who should be contacted for future permissions. These pastures are grazed by dairy cattle from mid-March to mid-September with a stocking rate of 2–6 animal units ha^{-1} depending on the season, and spread with cow manure each year. Plant cover consisted mainly in *Festuca elator L.*, *Phleum pratense*, *Trifolium repens L.*, and *Lolium*. The seventh population was located 35 km away from Yvetot (IR), near the University of Rouen in a location for which no specific permission was required (Fig. 1 and Table 1 for GPS coordinates). This field study did not involve endangered or protected species.

Individuals were preserved in pure ethanol for DNA analysis. Total genomic DNA was extracted from a segment of the anterior end of the earthworm using the CTAB extraction protocol: digestion using proteinase K, followed by protein precipitation with CTAB, a chloroform:isoamyl alcohol (24:1) wash and DNA precipitation with sodium acetate (3 M) and ethanol.

Mitochondrial DNA amplification and sequencing

A fragment of the *cytochrome c oxidase subunit I* mitochondrial gene (COI) was amplified and sequenced using the universal primers LCO1490 and HCO2198 [34]. For the I20 and IR populations, sequences were taken from Torres-Leguizamon et al. [27] (GenBank accession numbers JN381881–JN381930). Each amplification mixture (25 µl) contained 10 ng DNA, 12.5 µl of Taq PCR Master Mix (Qiagen, Hilden, Germany) and 0.25 µM of each primer. Polymerase chain reactions were performed using an initial denaturation step at 94°C for 3 min, followed by 40 cycles of the three following steps: denaturation at 94°C for 30 s, annealing at 49°C for 1 min and extension at 72°C for 1 min 30 s. The final extension was done at 72°C for 10 min. PCR products were purified using Microclean (Microzone Limited, Haywards Heath, UK). Both strands of amplicons were sequenced using BigDye Terminator Cycle sequencing kit version 1.1 (Applied Biosystems, Foster City, CA, USA) according to the standard protocol used in the genomic platform at the Mondor Institute of Biomedical Research (Créteil, France). Sequences were deposited in GenBank (Accession numbers: KF856627–KF856710).

Mitochondrial DNA sequence analysis

COI sequences were aligned manually using BioEdit v. 7.0.5.3 [35]. For each population, haplotype diversity (h) and nucleotide diversity (π) were estimated using DNAsp v. 5.10 software [36]. Haplotypic richness after rarefaction to a population size of 11 individuals was estimated using Contrib software [37]. Departure from neutrality was tested using Fu's *Fs* [38] and Ramos-Onsins and Rozas' *R2* statistic [39], which are powerful tests for detecting recent population expansion under assumptions of neutrality. *R2* is appropriate for small sample sizes [39]. The significance of *R2* and *Fs* were evaluated by comparing their observed values with their null distribution, generated by 10 000 random replicates using the empirical population sample size and observed number of segregating sites implemented by DnaSP v. 5.10. [36].

To describe the phylogenetic relationships between haplotypes, a statistical parsimony network was constructed using TCS v. 1.21 [40]. The divergence among haplotypes was calculated in MEGA 5 using the mean uncorrected p-distance [41].

To investigate the regional structure of *A. icterica* populations at the mitochondrial level, the overall genetic differentiation between populations was first estimated by calculating the global Φ_{st} in Arlequin v. 3.5.1.2 software [42]. We then performed a spatial analysis of molecular variance using SAMOVA software [43]. Clusters are identified based on geographic proximity and genetic homogeneity [43]. The geographic coordinates of each sampled locality were used as spatial information. Simulations were conducted with 'K' (number of groups) ranging from two to seven and each simulation annealing process was repeated 100 times.

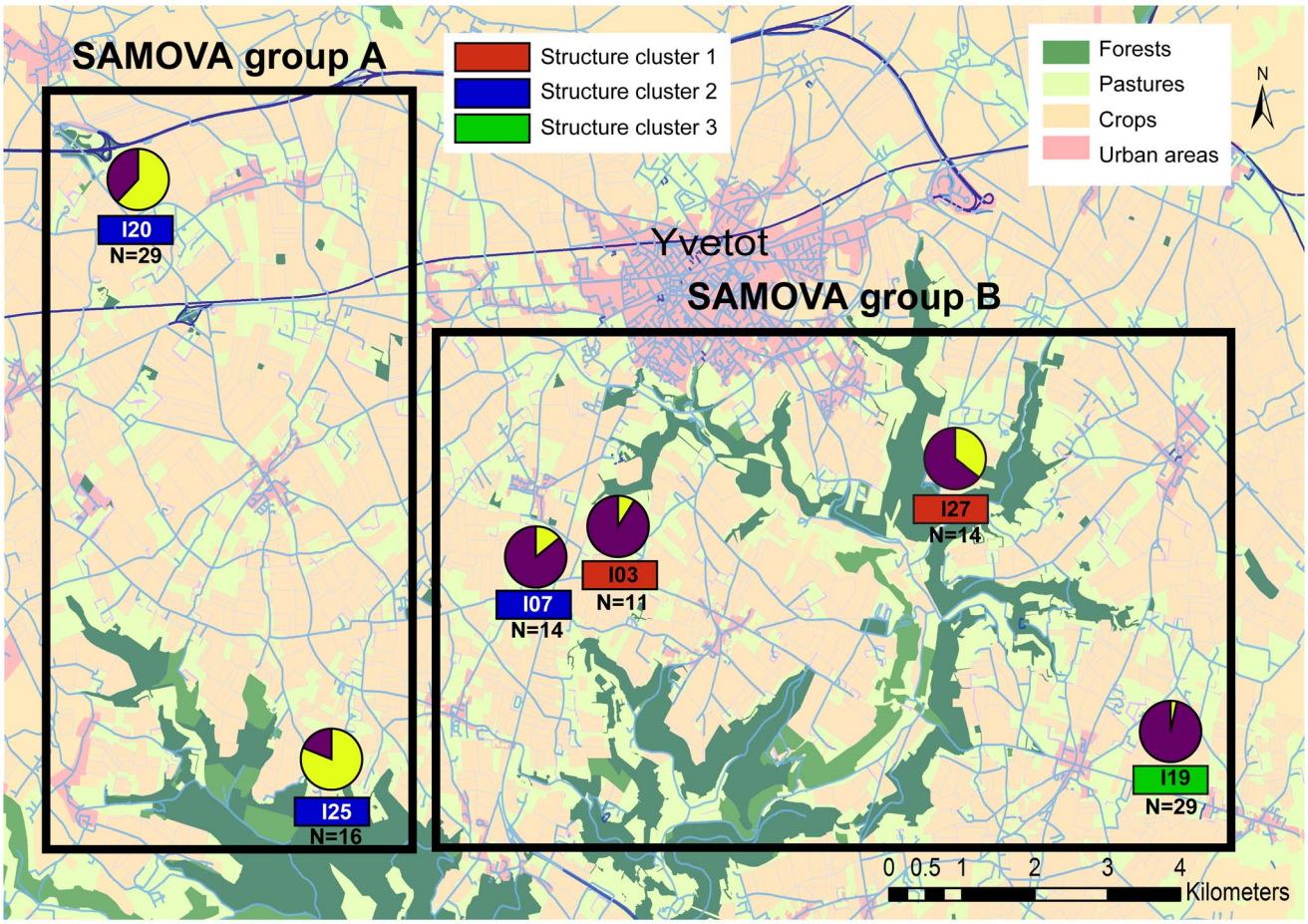

Figure 1. Geographical distribution of Yvetot populations and repartition of *cytochrome oxidase subunit I gene* (COI) lineages. Lineage 1 (L1) is shown in yellow and Lineage 2 (L2) is shown in purple. Groups revealed by the SAMOVA analysis of mitochondrial data and STRUCTURE analysis of microsatellite data are shown. Land use is also indicated.
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The clustering giving the highest Φ_{ct} value, corresponding to the optimal number of groups, was selected.

Microsatellite genotyping

Individuals were genotyped using seven microsatellite loci: Ai45, Ai56, Ai68, PB10D, 2PE40, 2PE70, C4 [27]. For individuals from the I20 and IR populations, genotypes were taken from Torres-Leguizamon et al. [27]. The seven loci were amplified by a touchdown PCR procedure that included an initial denaturation step of 3 min at 94°C, followed by 35 s at 94°C, 45 s at the initial temperature T_a +8°C, 10 cycles in which the temperature was decreased by 1°C per cycle, 1 min at 72°C, 25 cycles of 35 s at 94°C (except for PB10D and C4 for which 30 cycles were done), 45 s at Ta −2°C, 1 min at 72°C, with a final elongation step of 10 min 72°C. Each amplification mixture (15 µl) contained 10 ng/µl DNA, 1X reaction buffer (GoTaq Flexi buffer 5X), 2.5 mM of $MgCl_2$ (except for Ai56 and Ai68 for which 1.5 mM was used), 0.5 µM dNTPs, 0.25 µM of each primer and 0.5 units of GoTaq Flexi DNA polymerase (Promega, Madison, WI, USA). PCR products were loaded on an ABI 310 sequencer along with the LIZ500 size standard; alleles were scored using Genescan software (all from Applied Biosystems, Foster City, CA, USA).

Statistical analysis of microsatellite variation

In each population, the genetic diversity was analysed by computing allelic frequencies, number of alleles (N_{all}) and unbiased expected heterozygosity (H_e) using Genepop v. 4 software [44]. To take into account differences in sample size, allelic richness (A) after rarefaction to a population size of 22 individuals was estimated using FSTAT v. 2.9.3. software [45]. Exact tests for genotypic linkage disequilibria and deviations from Hardy-Weinberg equilibrium (HWE) were computed using Genepop v. 4 [44]. The sequential Bonferroni method was applied to adjust for multiple comparisons. Weir and Cockerham's (1984) estimator of the inbreeding coefficient F_{is} was calculated using Genepop v. 4 [44]. The presence of null alleles was tested using Micro-Checker v. 2.2.3 software, in which the Oosterhout method [46] was implemented and potential frequency of null alleles was estimated.

We tested for deviation from mutation-drift equilibrium in the study populations using the approach detailed in Cornuet & Luikart [47] and implemented in their software BOTTLENECK v. 1.2.02. Using a Wilcoxon test, observed heterozygosity was compared with the heterozygosity expected under equilibrium considering a two-phase mutation model (TPM) recommended for microsatellite data [48]. Recently founded populations are expected to show a transient excess of expected gene diversity, whereas expanding populations (e.g. recovering from a bottleneck)

Table 1. Genetic diversity in *A. icterica* populations.

				Mitochondrial data					Microsatellite data				
Locality	**Code**	**Latitude (N)**	**Longitude (E)**	N_{mt}	N_h	$r_{(11)}$	h	π	N_{ms}	N_{all}	$A_{(22)}$	H_e	F_{is}
Yvetot	**I03**	49.589	0.745	11	2	1.000	0.182	0.021	28	2.57	2.54	0.339	0.112
	I07	49.586	0.730	14	3	0.967	0.264	0.032	24	3.86	3.84	0.515	**0.386**
	I19	49.568	0.858	29	3	0.759	0.135	0.009	43	4.14	3.80	0.550	**0.379**
	I20	49.628	0.648	29	2	0.999	0.488	0.053	33	2.57	2.48	0.395	**0.176**
	I25	49.564	0.687	16	5	2.896	0.742	0.042	31	3.57	3.41	0.513	**0.232**
	I27	49.606	0.815	14	4	2.571	0.626	0.060	34	3.43	3.32	0.514	**0.372**
Rouen	**IR**	49.459	1.077	21	8	3.060	0.600	0.048	25	3.14	3.14	0.497	**0.442**
Total				134	15	-	0.563	0.041	218	5.71	-	0.561	**0.419**

Sample locations and codes are given, together with mitochondrial data: sample size (N_{mt}), number of haplotypes (N_h), haplotypic richness after rarefaction to a population size of 11 ($r_{(11)}$), haplotype diversity (h) and nucleotide diversity (π), and microsatellite data: sample size (N_{ms}), mean number of alleles (N_{all}) allelic richness after rarefaction to a population size of 22 (A_r), expected heterozygosity (H_e), estimator of the inbreeding coefficient F_{is} (significant values are in bold).

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or populations resulting from immigration from differentiated sources should show a deficit in expected gene diversity [47].

To investigate the genetic structure among populations, a G-test of allelic differentiation was carried out using Genepop v. 4 [44]. In addition, we performed a principal component analysis (PCA) on gene frequency data using PCAGen v. 1.2.1 software (available at http://www2.unil.ch/popgen/softwares/pcagen.htm). The significance of each principal component was assessed from 1000 permutations. We also used the software STRUCTURE v. 2.3.1 [49] to estimate the number of genetic clusters (K) present among all populations. This software generates clusters of individuals based on the Hardy-Weinberg model of genotypic distribution. Simulations were run using the admixture model without prior population information. We modelled cluster assignments for K ranging from 1 to 10. We performed 25 independent runs for each K value to confirm consistency across runs. In all simulations, we applied a burn-in period of 10 000 iterations and 100 000 Markov chain Monte Carlo iterations. To determine the most likely value of K, we used the ΔK method [50].

To estimate recent migration rates and test for significant cases of assignment to populations other than the population of origin (i.e. first-generation dispersers) we used the Bayesian method [51] implemented in GeneClass2 v. 2.0. [52] paired with a Monte Carlo resampling method for computation of assignment probabilities for each population [53] using 10 000 simulated individuals.

To test for the null hypothesis of independence between genetic and geographic distances, the logarithm of Euclidian geographic distances were plotted against $F_{st}/(1-F_{st})$ to compute a linear relationship following the recommendations of Rousset [54] and Mantel tests [55] were performed using Genepop v. 3.4 [44] across 100 000 permutations.

Cytonuclear disequilibrium analysis

Departures from random cytonuclear associations were tested using the CNDm programme [56]. The analyses were carried out by encoding mitochondrial haplotypes as two synthetic lineages (L1 and L2). Normalised cytonuclear disequilibria (CND) were calculated following Asmussen & Basten [33] for allelic associations, and significance levels were tested using Fisher's exact test.

Results

mtDNA genetic variation

The amplified COI fragment contained a homopolymer poly-C. In most of the reactions, the sequence became mixed after the poly-C, most probably because of polymerase stutter. Sequences were thus truncated (fragment length <200 bp). Consensus sequences shorter than 374 bp were excluded of the analysis. Over the whole mtDNA data set (134 sequences), we detected 15 haplotypes defined by 34 parsimony informative sites (9%) among 44 variable sites (12%). Within populations, haplotype diversity (h) ranged from 0.135 to 0.742 and nucleotide diversity (π) ranged from 0.00864 to 0.05973 (Table 1). Populations displaying low haplotypic richness were I03, I07, I19 and I20 ($r_{(11)} \leq 1$) while I25, I27 and IR showed relatively high values of haplotypic richness (2.5 to 3). None of the Fu's *Fs* and *R2* values were significant.

The haplotype distribution at the population level is shown in Figure 2. This haplotype network illustrates the relationships between the 15 haplotypes and shows a clear separation of *A. icterica* haplotypes into two divergent lineages L1 and L2 (Figs. 1 and 2). Both lineages showed a high percentage of divergence (8.7%). L1 consisted of 47 individuals and 8 haplotypes, two of which were abundant (H1 and H2). Within L1, haplotypes were

more divergent (i.e. separated by several mutational steps) than within L2. L2 included sequences from 87 individuals and the most abundant haplotype (H5) was found in all populations, representing over half the L2 individuals (60.5%). The remaining seven L2 haplotypes were relatively infrequent but all closely related. They differed from the most common haplotype H5 by at most only two mutation steps.

Φ_{st} analysis showed significant genetic structure at the level of the whole study (7 populations, $\Phi st = 0.324$, $p<0.001$). In terms of regional mitochondrial structure, the SAMOVA showed that the highest significant value ($\Phi_{ct} = 0.409$) was obtained when the populations were split into two groups (Fig. 1): group A corresponded to the I20 and I25 populations and group B was composed of the remaining populations in the Yvetot area (I03, I07, I27, I19) and the IR population (not shown in Fig. 1).

nDNA genetic variation

Among the seven microsatellite loci, the number of alleles per locus ranged from one to seven (Table 2). None of the loci showed significant linkage disequilibrium. Genetic diversity indices varied among populations (Table 1), with the I03 and I20 populations showing lower values ($A = 2.54$, $H_e = 0.339$ and $A = 2.48$, $H_e = 0.395$, respectively) than the other populations ($3.14<A<3.84$ and $0.497<H_e<0.515$). Depending on the population, the Hardy-Weinberg expectations (HWE) test showed a significant deviation for some of the loci (Ai68, Ai56, C4, PB10D and 2PE70). However, null alleles were suspected for several loci (Table 2). The estimated frequencies of null alleles ranged from 12.1% (locus 2PE70, population I19) to 40.9% (locus C4, population I19). The data set was thus corrected for null alleles and both data sets (original and corrected) were used for analyses based on allelic frequencies.

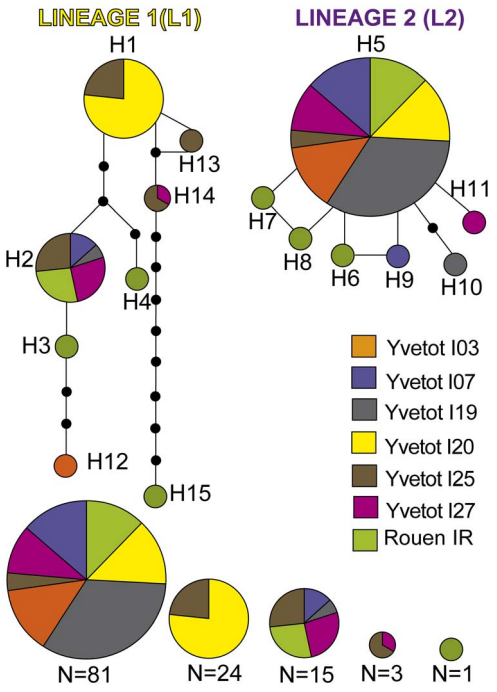

Figure 2. ***Cytochrome oxidase subunit I*** **gene (COI) haplotype 95% statistical parsimony network for Yvetot and Rouen** ***A. icterica*** **samples.** H1 and H5 represent presumed ancestral sequence. Circle size is relative to the proportion of each haplotype in the sample. Mutational steps are indicated by small black circles.
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Table 2. Hardy-Weinberg equilibrium P-value (significant values after sequential Bonferroni correction are in bold) together with the estimation of null allele frequency (in parentheses) for *A. icterica* microsatellite markers in each study populations.

	Locus ID (Number of alleles)						
Population	**Ai45 (4)**	**Ai56 (7)**	**Ai68 (8)**	**PB10D (4)**	**2PE40 (3)**	**2PE70 (3)**	**C4 (11)**
I03	0.296	1.000	1.000	0.077	ML	0.442	0.093 (0.155)
I07	0.500	0.609	0.067 (0.146)	**0.001** (0.256)	ML	**0.001**	**0.000** (0.385)
I19	0.116	0.459	0.002 (0.216)	0.330	0.144	0.013 (0.121)	**0.000** (0.409)
I20	0.105	ML	0.755	0.935	ML	**0.001** (0.274)	**0.000** (0.252)
I25	0.031	0.405	0.463	0.110	ML	0.002 (0.257)	**0.000** (0.314)
I27	0.613	0.217	**0.000** (0.182)	**0.000** (0.210)	ML	0.163	**0.000** (0.346)
IR	0.315	**0.000** (0.318)	0.002 (0.230)	1.000	1.000	0.002 (0.284)	**0.000** (0.306)

ML: monomorphic locus.
doi:10.1371/journal.pone.0101597.t002

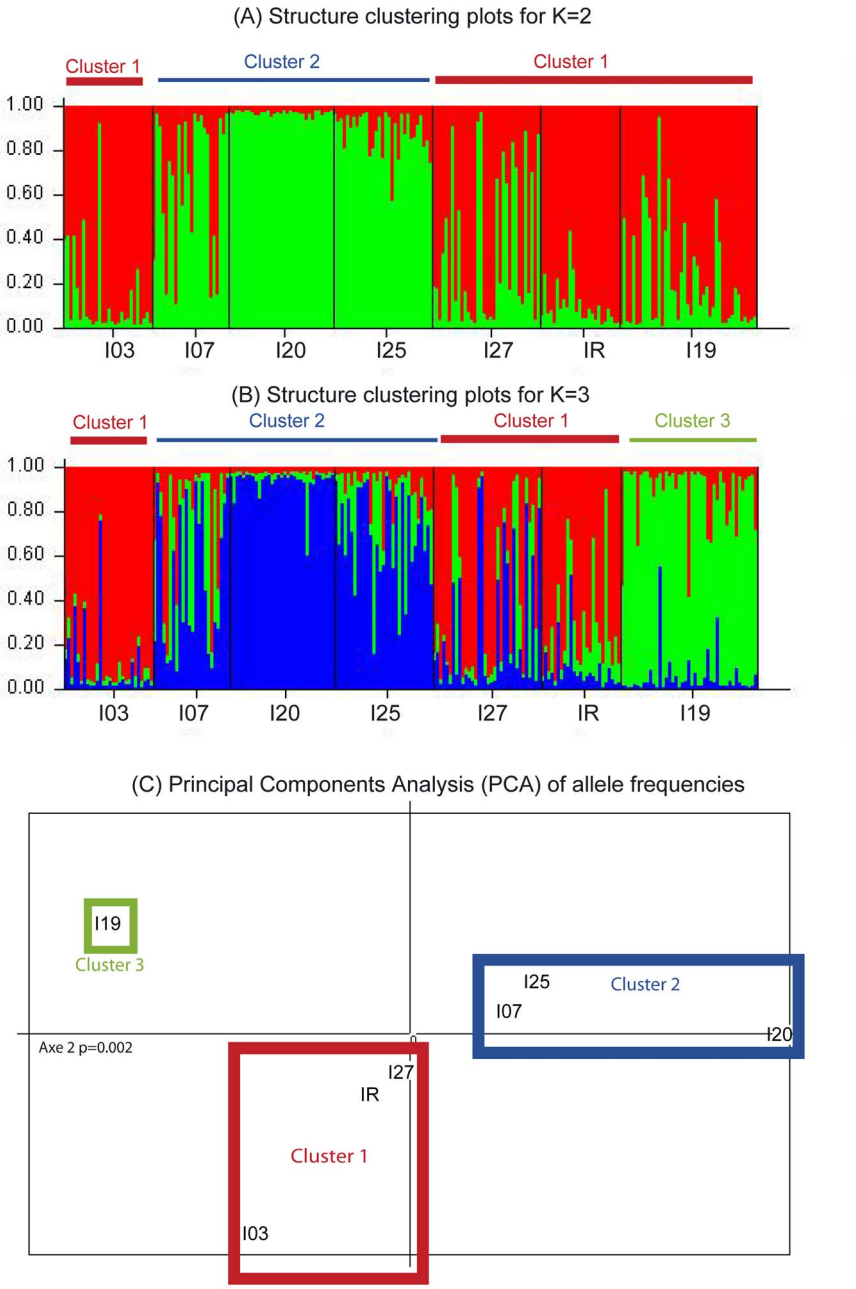

Figure 3. Genetic clustering of *A. icterica* populations based on analysis of microsatellite data. A and B. STRUCTURE Q plots representing the number of genetic nuclear groups for K = 2 and K = 3 respectively in *A. icterica* for I03, I07, I20, I25, I27 and IR populations. Each individual is represented by a vertical bar showing degree of admixture. C. Principal components analysis (PCA) of microsatellites allele frequencies for the whole dataset. Level of significance was derived from 1000 permutations and significant P-value is shown.
doi:10.1371/journal.pone.0101597.g003

When testing for mutation-drift equilibrium, a significant gene diversity excess was detected only in the I27 population using the original dataset, but also in the I07, I20, I25 and IR populations using the corrected dataset without null alleles, suggesting that these populations are good candidates for recent demographic disequilibrium arising from a population bottleneck.

Significant genetic structure was revealed at the level of the whole study (G-test, $p<0.001$). No pattern of isolation by distance was observed among the six Yvetot populations ($p = 0.342$ and $p = 402$ using the original and the corrected data set respectively). Clustering analysis (Figs. 3A and 3B) clearly indicated genetic similarities among the I03, I27 and IR populations (Cluster 1) and among I07, I20, I25 populations (Cluster 2). The case of I19 was more ambiguous. The highest ΔK value was obtained for $K = 2$ ($\Delta K = 60.90$, Fig. 3A), although the ΔK value for $K = 3$ was comparable ($\Delta K = 47.87$, Fig. 3B). For $K = 2$, the I19 population was grouped with Cluster 1, whereas for $K = 3$, it formed a third group. The results of the PCA on allelic frequencies were in agreement with the results of the clustering analysis (Fig. 3C). The populations were separated into two major groups along the second axis of the PCA, which was highly significant (original dataset, $p = 0.002$ and corrected data set, $p = 0.001$). Population

Table 3. Inference of gene flow between populations belonging to each group defined using Structure: the percentage of individuals assigned to each locality as estimated by GeneClass2 is presented.

	Cluster 1			Cluster 2			Cluster 3	
	I03	I27	IR	I07	I20	I25	I19
I03	82	3	12	0	0	0	0
I27	7	68	36	25	3	6	2
IR	7	6	44	0	0	0	2
I07	0	15	0	42	3	16	2
I20	0	0	0	8	82	6	0
I25	0	3	0	21	9	65	0
I19	4	6	8	4	3	6	93

Source localities are given in rows, recipient localities in columns.
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I19 was genetically isolated from the two clusters. Within each group, I03 and I20 populations were highly differentiated from the other two populations in their respective group (Fig. 3B).

Contemporary gene flow was detected at this regional scale with 64 individuals (29%) identified as first-generation dispersers. Among these 64 individuals, 30 belonged to Cluster 1, 25 belonged to Cluster 2 and the 9 remaining migrants belonged to the I19 population (Table 3). Most of the first-generation dispersers were assigned to populations belonging to the same cluster (19 first-generation dispersers from Cluster 1 out of 30 and 18 first-generation dispersers from Cluster 2 out of 25). Populations I03, I20 and I19 showed the lowest number of dispersers (5, 6, and 3 migrants, respectively).

Relationships between mitochondrial lineages and microsatellite alleles

The test for overall non-random association between microsatellite alleles and mitochondrial lineages revealed significant cytonuclear disequilibrium after Bonferroni correction for four of the most polymorphic microsatellite loci (Table 4). Three alleles showed significant association with L1, whereas 5 alleles were significantly associated with L2.

The test was also carried out within each population that showed both lineages, with the rarest representing at least 30% (IR, I20 and I27). The association between allele 129 at the Ai56 locus and L1 was suggested in the IR population, but was not significant after Bonferroni correction ($p = 0.037$). In addition, there was a trend for an association between allele 178 of the PB10 locus and L2 ($p = 0.092$) in the I20 population.

Discussion

Low genetic diversity within A. icterica populations

The level of polymorphism detected in *A. icterica* populations using microsatellite markers and COI sequences was surprisingly low (Table 5 and Table 6). The seven microsatellite markers showed low genetic variability with only 3 to 11 alleles over all loci. This polymorphism was lower than that reported in all other microsatellite datasets on earthworm populations (Table 5). For instance, the mean number of alleles per locus (N_A) ranged from 2.57 to 4.14, but values of 5 to 17 alleles have been reported in other earthworm species (*Eisenia fetida* [57] and *Lumbricus terrestris* [58], respectively). Similarly, the sequenced fragment of the COI gene (374 bp) displayed low genetic variability in comparison to other earthworm species, despite the relatively restricted geographical scale and the short length of sequenced fragment tested in this study. For instance, only 12% of sites were polymorphic, but 33% (*Hormogaster elisae* [30]) to 36.5% (*Metaphire sieboldi* [59]) of sites are polymorphic in other earthworm species.

Ancient bottleneck events due to population isolation in periglacial refugia may be partly responsible for the current low genetic variation in this earthworm species. Among contemporary events, there are two major explanations for the low level of polymorphism in *A. icterica*: the occurrence of recent population bottlenecks and/or recurrent inbreeding due to reproduction between relatives. High inbreeding due to preferential mating among relatives (see for instance [60]) is unlikely since deviation from HWE was inconsistent across loci and populations and could be attributed to null alleles. In some *A. icterica* populations, inbreeding may nevertheless occur due to a decline in effective population size. Our results indeed suggested that some populations were recovering from a recent population bottleneck. Bottlenecks can occur following colonisation events because the number of initial colonists is often small and genetic drift may

Table 4. Cytonuclear linkage disequilibrium between *A. icterica* mitochondrial lineages and microsatellite alleles, estimated using CNDm software (Basten & Asmussen 1997).

Microsatellite locus (Number of alleles*)	mtDNA lineage	
	L1	L2
Ai45 (3)	-	-
Ai56 (5)	129	133
Ai68 (8)	-	118
PB10D (4)	220	178, 182
2PE40 (3)	-	-
2PE70 (3)	-	-
C4 (9)	176	178

Alleles significantly associated with mitochondrial lineage, after Bonferroni correction, are indicated.
* the analysis was only executed for samples for which both COI haplotypes and multilocus microsatellite genotypes were scored.
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result in reduced genetic variation in a newly established population [17]. However, in an outcrossing species such as *A. icterica*, the likelihood of a genetic bottleneck is low because even only a few immigrants can introduce a large increase in genetic variation [61]. Only successive and drastic bottlenecks could have severely affected the genetic variation of *A. icterica*. Agricultural practices such as crop rotation can contribute to a fragmentation of the species habitat and thereby cause successive genetic bottlenecks [17]. In addition, geographic isolation of populations due to natural and artificial barriers to gene flow can accentuate the loss of genetic variability through genetic drift. Low levels of gene flow in fragmented habitats can even lead to extinction of local populations due to stochastic processes. Extinction-recolonisation is a classic metapopulation scenario, with periodic extinction of all individuals in a particular patch and subsequent recolonisation of this patch from surrounding areas [17].

Relationship between genetic and geographic distances at a regional scale

A relatively high level of genetic differentiation was revealed among localities, regardless of the marker used. Interestingly, no relationship between genetic and linear geographic distances was observed at this regional scale (populations separated by less than 13 km in the Yvetot area), corroborating other earthworm population genetics studies (reviewed in [14], but see [30]). The lack of relationship between genetic differentiation and geographic distances was confirmed by the cluster analyses. At the nuclear level, populations were clustered into two major groups (Cluster 1 = I03, I27 and IR and Cluster 2 = I07, I20, I25), within which most of the first-generation dispersers were detected. It is noteworthy that two geographically close populations (I03 and I07) did not belong to the same cluster. There are two hypotheses that can explain the lack of correlation between the genetic and geographic distances. First, stochastic events, such as environmental changes, demographic factors (i.e. chance differences among individuals in survival or fecundity) and genetic drift may be more important than active dispersal in partitioning genetic variation at this scale (i.e. 1 to 15 km). Among earthworms, which are believed to be able to actively disperse at distances ranging from 4 to 14 m $year^{-1}$ (review in [11,62]), *A. icterica* is considered to be relatively vagile, being able to travel up to 500 m $year^{-1}$ under conditions that trigger dispersal [11,24]. Tracing active dispersal events requires a study at a finer scale (<500 m^2).

Second, passive dispersal due to rain, floods, streams, birds, cattle or various human activities [62] may promote gene flow between geographically distant populations. In agricultural regions, such as in the Yvetot area, earthworms or cocoons are likely to be passively dispersed via various human activities that involve transporting soil or plant material, for instance (see [62] for review).

Discordant patterns of mitochondrial and microsatellite genetic structure

Two divergent (8.7%) mitochondrial lineages were observed within studied populations of *A. icterica*. In the Yvetot area, most of the individuals from the I20 and I25 populations belonged to L1 whereas the majority of samples from I03, I07, I19 and I27 belonged to L2 (Fig. 1). In Rouen, the population was predominantly composed of individuals belonging to L2. Genetic differentiation was confirmed in the SAMOVA analysis, with a grouping along the same lines.

Divergent sympatric mitochondrial lineages often reveal the existence of cryptic species, particularly when divergence is confirmed in the nuclear compartment of the genome and/or when reproductive isolation between lineages has been demonstrated [29,63]. In *A. icterica*, our results indicate that the two divergent lineages were randomly interbreeding with respect to mtDNA haplotypes over a relatively restricted geographical area. Deep mtDNA divergence despite clear interbreeding can reflect long periods of geographical isolation followed by secondary contact favouring gene flow, homogenising the nuclear genome over time.

Glaciation, which became increasingly severe throughout the Pleistocene, is known to have drastically modified species distributions [64]. Most organisms presently distributed across Europe retreated to refugia during glaciation ca. 18 000 years BP, mostly in the peninsulas of Iberia, southern France, Italy and the Balkans, and, in some cases, near the Caucasus and the Caspian Sea [64]. Although no common phylogeographic histories across Europe have been proposed, Taberlet et al. [65] highlighted some concordance in two postglacial colonisation routes: (1) from Iberia and southern France towards Scandinavia and (2) from a Balkan refugium towards south-eastern France. Recent analyses of earthworm communities have shown that past climate changes have left a deep footprint on present-day earthworm diversity patterns, from community to macroecological scales [66]. It appears that earthworms recolonised France from two large

Table 5. Polymorphism of microsatellite loci in earthworm species.

Morphospecies	Sampling design	Geographical range	N_{ind}	N_{loci}	N_A	H_e	Reference
Allolobophora chlorotica	2 populations in 2 countries	~500 km	62	8	6.63–9.63	0.725–0.774	[63]
Aporrectodea icterica	7 populations in northern France	<100 km^2	218	7	2.57–4.14	0.339–0.550	This study
Aporrectodea longa	1 site	<0.50 km^2	31	11	7.18	0.654	[73]
Eisenia fetida	3 vermiculture stocks	NA	70	16	5.00–5.75	0.630–0.660	[57]
Hormogaster elisae	1 site	<0.50 km^2	26	10	12.5	0.821	[74]
	1 site	0.064 km^2	75	4	7.32	0.890	[75]
Lumbricus rubellus	1 site	<0.50 km^2	34	8	9.75	0.669	[76]
Lumbricus terrestris	1 site	<0.50 km^2	32	10	12.8	0.853	[77]
	1 site	<0.50 km^2	281	3	17	0.817	[58]

For each earthworm species for which microsatellites have been used for population genetics study, the number of polymorphic loci (N_{loci}), mean number of alleles per locus (N_A), and multilocus gene diversity (H_e) are indicated.
NA: information not provided in the study.
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Table 6. COI sequence polymorphism in earthworm morphospecies.

Morphospecies	Number of locations	Sampling area	Geographical range	N_{ind}	L_{seq}	N_h	P_S (%)	N_L	Reference
Allolobophora chlorotica	38	5 European countries	~430 000 km^2	153	582	54	NA	7	[63]
Aporrectodea icterica	7	Northern France	<100 km^2	134	374	15	12	2	This study
Aporrectodea trapezoides	47	11 countries	~5 510 000 km^2	178	456	37	34	2	[78]
Dendrobaena octaedra	6	Southern Finland	~52 000 km^2	118	441	24	NA	1	[79]
Hormogaster elisae	7	Central Iberian Peninsula	<100 km^2	82	658	38	33	6	[30]
Metaphire sieboldi	64	Southern Japan	~300 000 km^2	71	690	NA	36.5	1	[59]
Rhinodrilus alatus	21	Southeastern Brazil	~16 000 km^2	69	593	59	34	6	[80]

For each earthworm morphospecies for which COI has been recently used (since 2009) in a population genetics study, the length of sequence alignment (L_{seq}), the number of haplotypes (N_h), proportion of variable sites (P_S) and the number of divergent lineages (N_L) are indicated.
NA: information not provided in the study.
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refugia in southern France. However, there has been also recolonisation from eastern Europe and north-eastern France, and two micro-refugia were probably conserved in very specific locations in the Vosges (north-eastern France) and in Brittany (north-west of France). However, these recolonisation sources are difficult to assess [66]. Nevertheless, we can assume that the divergent *A. icterica* lineages originated from distinct refugia and that they merged during post-glaciation recolonisation. MtDNA divergence may thus be the result of neutral differences within the species, representing a historical mark of divergent lineages that have remerged [67,68]. Over time, haplotypes are lost due to genetic drift (i.e. lineage sorting), resulting in populations monophyletic for a single gene lineage [69]. Deep mtDNA divergence can only be maintained in a panmictic population with large effective population size, which effectively slows lineage sorting [68]. However, we argue that *A. icterica* has small effective population size and has undergone serial population bottlenecks during the process of post-Pleistocene recolonisation in northern Europe, further accentuated by recent bottlenecks due to habitat fragmentation. We therefore assume that *A. icterica* lineages have come into contact too recently for lineage sorting to be completed. Furthermore, human activities could be, at least in part, responsible for the merging of two divergent lineages. For instance, the contribution of historical human activities to the current pattern of spatial genetic structure was documented for numerous plant species (e.g. [70,71,72]). Overall, our results suggest both past and ongoing anthropogenic impacts on *A. icterica* population genetic structure.

Here, we investigated whether the process of remerging can be traced back by studying the cytonuclear disequilibrium within contemporary populations of *A. icterica*. In the global dataset, some nuclear alleles were non-randomly associated with one of the two mitochondrial lineages. Because of the low number of populations displaying enough copies of both lineages and because of the relatively low number of individuals for which both COI haplotypes and microsatellites genotypes were scored, this non-random association could not be confirmed at the population level. Thus we cannot completely exclude the possibility that the observed cytonuclear disequilibrium is due to genetic structuring at the scale of the study.

Conclusions

Overall, this study confirmed general patterns of genetic variation observed in earthworms, such as (i) the importance of historical events for explaining their current genetic variation and (ii) the weak relationship between genetic and geographic distances suggesting the importance of passive dispersal in structuring earthworm populations. Nevertheless, some uncertainties persist such as the underlying cause of the mito-nuclear discordance and the respective roles of active *versus* passive dispersal in partitioning population genetic structure. In particular, it is critical to investigate how individual dispersal interacts with landscape structure. Further study is now needed to examine the extent to which barriers to movement and corridors that facilitate dispersal determine earthworm population connectivity in heterogeneous landscapes.

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Author Contributions

Conceived and designed the experiments: LD JM MTL. Performed the experiments: MTL. Analyzed the data: LD MTL. Contributed reagents/materials/analysis tools: LD JM TD. Wrote the paper: MTL LD JM TD.

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Annexe 3

Mathieu, J., J.-P. Rossi, P. Mora, P. Lavelle, P.F.d.S. Martins, C. Rouland, and M. Grimaldi, Recovery of soil macrofauna communities after forest clearance in Eastern Amazonia, Brazil. Conservation Biology, 2005. 19(5): p. 1598-1605.

Recovery of Soil Macrofauna Communities after Forest Clearance in Eastern Amazonia, Brazil

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Abstract: *As primary forest is cleared, pastures and secondary forest occupy an increasing space in the Amazonian landscape. We evaluated the effect of forest clearing on a soil macrofauna (invertebrate) community in a smallholder farming system of southeastern Amazonia. We sampled the soil macrofauna in 22 plots of forest, upland rice fields, pastures, and fallows of different ages. In total, we collected 10,728 invertebrates. In cleared plots the species richness per plot of the soil macrofauna fell from 76 to 30 species per plot immediately after forest clearance, and the composition of the new community was different. Ants, termites, and spiders were most affected by the disturbance. In plots deforested several years before, the effect of forest clearance was highly dependent on the type of land use (pasture or fallow). In fallows, the community was similar to the initial state. The species richness per plot in old fallows rose to 66, and the composition was closer to the primary forests than to the other types of land use. On the contrary, in the pastures the species richness per plot remained low at 47. In fallows, all the groups showed a richness close to that in primary forest, whereas in the forest only the richness of earthworms and Coleoptera recovered. Our results show that forest clearing constitutes a major disturbance for the soil macrofauna and that the recovery potential of the soil macrofauna after 6 or 7 years is much higher in fallows than in pastures. Thus, fallows may play a crucial role in the conservation of soil macrofauna.*

Key Words: biodiversity, deforestation, smallholders, soil recovery potential

Recuperación de Comunidades de Macrofauna del Suelo Después de la Tala de Bosques en la Amazonía Oriental, Brasil

Resumen: *A medida que el bosque es talado, los pastizales y la vegetación secundaria cada vez ocupan más espacio en el paisaje Amazónico. Evaluamos el efecto de la tala del bosque sobre una comunidad de macrofauna (invertebrados) del suelo en un sistema agrícola de pequeña propiedad en el sureste de la Amazonía. Muestreamos la macrofauna en 22 parcelas de bosque campos de arroz, pastizales y barbechos de diferentes edades. En total, recolectamos 10,728 invertebrados. En parcelas taladas, la riqueza de especies de macrofauna del suelo por parcela disminuyó de 76 a 30 especies por parcela inmediatamente después de que el bosque fue talado, y la composición de la comunidad nueva fue diferente. Las hormigas, termitas y arañas fueron las más afectadas por la perturbación. El efecto de la tala del bosque fue altamente dependiente del tipo de uso de suelo (pastizal o barbecho) en las parcelas deforestadas varios años antes. En los barbechos, la comunidad fue similar al estado inicial. La riqueza de especies por parcelas en los barbechos viejos se elevó a 66, y la composición fue más cercana a la de bosques primarios que a la de los otros tipos de uso de suelo. Por el*

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contrario, la riqueza de especies por parcela en pastizales permaneció baja en 47. En los barbechos, todos los grupos mostraron una riqueza similar a la del bosque primario, mientras que en el bosque solo se recuperó la riqueza de lombrices y Coleópteros. Nuestros resultados sugieren que la tala de bosques constituye una perturbación mayor para la macrofauna del suelo y que el potencial de recuperación de la macrofauna del suelo después de 6 o 7 años es mucho mayor en los barbechos que en los pastizales. Por lo tanto, los barbechos pueden jugar un papel crucial en la conservación de la macrofauna del suelo.

Palabras Clave: biodiversidad, deforestación, pequeños propietarios, potencial de recuperación

Introduction

Natural succession greatly affects biodiversity and ecosystem functions. In Eastern Amazonia, characteristic successions take place in areas deforested by smallholders. The forest, cleared at the rate of 2 million ha every year in Amazonia (Laurance et al. 2001), is replaced by rice crops and then usually transformed into pastures or fallows (Parayil & Tong 1998). Many pastures are abandoned within 10 to 20 years because of loss of productivity (Costa & Rehman 1999; Desjardins et al. 2000; Alfaiai et al. 2004). This process drives the farmers to enlarge their grazing surface to compensate for the decreasing amount of food for cattle.

Conservation of the soil macrofauna may help keep land productive longer because these organisms maintain nutrient cycling and decomposition processes and modify the physical properties of soil (de Bruyn & Conacher 1990; Lavelle et al. 1997; Ekschmitt & Griffiths 1998). It is already known that soil macrofauna, and earthworms in particular, can lead to dramatic change in soil properties and plant productivity, especially in acid soils that often have low amounts of organic matter (Curry 1987; Lavelle et al. 1994; Chauvel et al. 1999). Little is known, however, about the recovery potential of soil macrofauna after forest clearance in Amazonia, especially in smallholder farming systems. In particular, if the role of fallows, or "secondary forests," in the recovery of many aboveground organisms (invertebrates and vertebrates) is recognized (Dunn 2004), the role of secondary forest in the conservation of soil invertebrates has been poorly investigated.

We quantified the effect of deforestation on the overall soil macrofauna community in a smallholder farming system of southeastern Amazonia. We also compared the recovery potential of the soil macrofauna among areas with different land uses. We identified the groups with the best recovery potential and discuss possible reasons for the differences in recovery potential among land uses.

Methods

Study Site

Benfica is a 10-year-old smallholder community that relies mainly on cattle ranching and rice production. The community is located in an area of current deforestation in eastern Amazonia (5°16′ S and 49°50′ E), near Marabà, State of Parà, Brazil.

The climate is tropical humid with annual rainfall of 1800 mm (wet season December to March) and an average temperature of 26° C. The landscape is fragmented and consists of small hills separated by a network of rivers and seasonally flooded land. Primary forest and pastures cover most of the area. Pastures are dominated by *Brachiaria bryzantha* (Staph) cv. Marandu sometimes mixed with *Panicum maximum* (Jacq.) cv. Tanzania. Seasonally flooded parts of the pastures are often dominated by *B. humidicola* (Rendle). The remaining space is in temporary rice fields, fallows, and family fruit orchards. Fallows are dominated by very fast-growing plants and look like secondary forests after 5 or 6 years. Clayey ferralsols (i.e., red and yellow weathered soils, whose colors result from an accumulation of metal oxides, formed on geologically old parent materials) are dominant in the study area (Deckers et al. 1998).

Reconstitution of the Chronological Sequence

In this area of Amazonia, a characteristic succession of land use is common to most smallholder farming systems. This kind of agricultural system is characterized by small exploitations (50 ha on average), no mechanization, and low use of insecticides or fertilizers. After deforestation by slash and burn, farmers generally establish rice fields for 1 or, less frequently, 2 years and then transform them into pastures or they are left as fallows.

To study the evolution of soil macrofauna during succession, we sampled 22 plots in different stages of the exploitation sequence: six primary forest plots, five rice fields (1 year old), one young fallow (2 years old), two young pastures (1 year old), four established pastures (6 years old), and four established fallows (7 years old). All the established pastures and fallows had been exploited as rice fields after forest clearance and had the same grazing history. Forest was cleared with slash-and-burn methods, and all pastures were burned annually in a prescribed fire at the end of the dry season. The 1-year-old pasture plots had never been grazed, and the grass cover was high, up to 2 m. Plots of the same type of land use were separated by at least 400 m or by a stream or by both.

In each plot we sampled a set of 10 to 25 points distributed at regular intervals along one to three 50-m-long transects. The number of transects was set so that all the different soil subtypes within the plots would be sampled because the soil type (ferralsol or cambisol) can influence diversity of local soil macrofauna in pastures at the plot scale (Mathieu et al. 2004). We sampled a total of 270 points.

Soil Macrofauna Sampling

At the end of the wet season in 2002, we sampled soil macrofauna (i.e., groups in which more than 90% of individuals are visible to the naked eye) according to the methods of Anderson and Ingram (1993). We focused on the most abundant, broad taxonomic groups of the soil macrofauna (i.e., earthworms, ants, termites, Coleoptera, spiders, chilopods, and diplopods). The samples were blocks of soil (25 × 25 × 30 cm deep) that were dug out quickly. We hand sorted the soil macrofauna and preserved most organisms in 75% alcohol. Earthworms were preserved in 4% formaldehyde. Litter macrofauna was also collected. All individuals were later sorted and identified at the species level with the help of taxonomists.

Statistical Analysis

Five standard indexes were used to describe the evolution of the community structure: Shannon diversity index, Simpson (inverse: 1/*D*) dominance index, frequency of the species, the species richness per sample, and the species richness per plot. We calculated the species richness per sample as the average number of species per sample for a given plot. The species richness per plot was calculated as the average number of species per plot for a given type of land use. Because the number of samples differed among the different types of land use, we used rarefaction methods (in ECOSIM [Gotelli & Entsminger 2001]) to calculate the expected values of the diversity indexes at the minimum number of sampling points per plot (10 samples per plot) (Simberloff 1972; James & Wamer 1982). The frequencies were calculated as the probability of the presence of each species in each plot.

To study the similarity of the soil macrofauna among plots, we analyzed community composition data with a hierarchical ascendant classification (HAC) based on chi-square metrics. This type of distance is asymmetrical (i.e., it does not consider double absence as a similarity between sites) (Legendre & Legendre 1998). Hierarchy was calculated using unweighted arithmetic average clustering (UPGMA), also called the "average link" algorithm. We used the matrix of the frequencies of each species in each plot to reduce the bias introduced by the presence of social insects, which can present locally extremely high densities. We used the ADE-4 program (Thioulouse et al. 1997) for classification. The differences were tested using multiple-mean comparisons with nonparametric tests (Kruskall and Wallis) followed by Mann-Wittney *U* tests (Sokal & Rohlf 1995). The data for the young fallow stage were not included in the tests because there was only one plot.

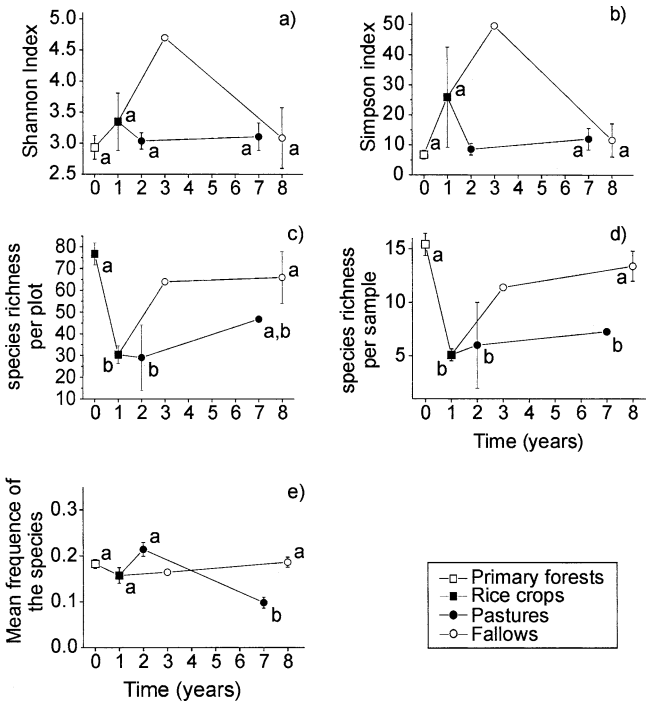

Figure 1. Diversity indices of the soil macrofauna at the different succession stages of smallholders farming systems: (a) Shannon index, (b) Simpson index, (c) species richness per plot, (d) species richness per sample, and (e) mean frequencies of the species per plot. Stages that do not have common letters are different ($p < 0.05$). *Error bars: standard error of mean.*

Results

Changes in Community Structure

The value of the Shannon diversity index was higher in the rice fields than in the primary forests (Fig. 1a), but this difference was not significant. In the primary forests the Shannon index was 2.9, whereas in rice fields it reached 3.3 on average, although it was variable. In pastures it remained fairly constant, from 1 year old ($H = 3.0$) to 6 years old ($H = 3.1$). The Shannon index was much higher in the young fallow ($H = 4.9$) than in the forest. In old fallows, however, it decreased to 3.1, which is fairly close to that of primary forests and pastures.

The Simpson index followed the variation in the Shannon index (Fig 1b). It increased from 6.8 in forests to 25.9 in rice fields. In 1-year-old pastures, the Simpson index was close to that of primary forests (i.e., 8.6) as were the

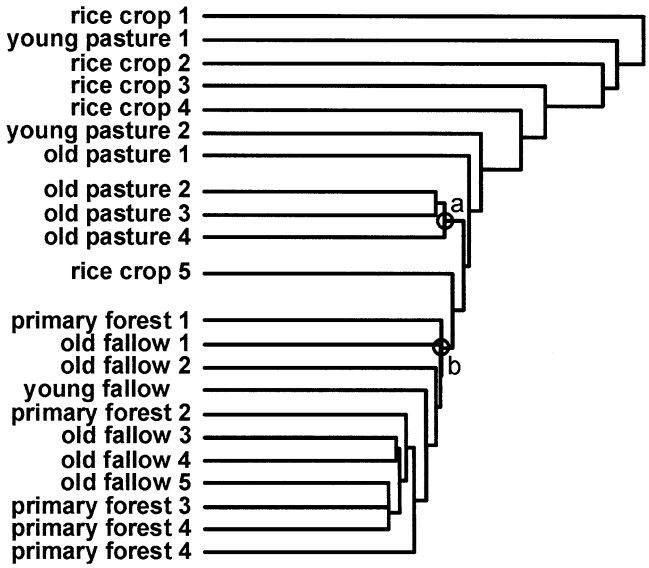

Figure 2. Classification of the plots according to their soil macrofauna community based on a hierarchical ascendant classification (HAC) (a, b are distinct clusters, see text).

Simpson indexes in the old pastures (12.0). In the young fallow, it was 49.6, much higher than in primary forests or rice fields. In the old fallows, however, it was 11.6, a value close to that in primary forests and in old pastures.

Species richness per plot in recently deforested plots was less than half that in primary forest (30 species per plot in rice fields vs. 76 in forests, Fig 1c). In old pastures, the species richness reached 47 species per plot. The species richness in young fallow was very high after 2 years: 64 species per plot, a value even higher than that in the primary forest. In old fallows, the species richness per plot was still high (66 species per plot).

The species richness per sample varied strongly with the type of land use (Fig. 1d) and was highest in the primary forests, with 15 species per sample on average. In 1-year-old deforested plots (rice fields), species richness dropped to 5 species per sample. In pastures richness remained low, reaching 7.2 species per sample in 6-year-old plots. In fallows, richness was much higher than in rice fields and pastures, reaching 11.4 species per sample in 2-year-old fallows and 13.4 species per sample in 7-year-old fallows, respectively.

Forest clearance had a limited effect on species frequencies (Fig. 1e). They changed from 0.18 in the primary forests to 0.15 in the rice fields. Frequencies were very similar in fallows (0.19) and primary forest. In old pastures, however, species frequencies (0.09) were lower than in all the other stages.

The dendrogram shows a strong separation between a cluster formed by the forest and fallow plots and the rest of the plots (Fig. 2, node b). Within the broad cluster, the forest and fallow plots were not well separated, and the difference between these plots was never high. Outside this cluster nearly all plots were isolated on single branches. Three of the four old-pasture plots formed a distinct cluster (node a). The remaining plots did not form any cluster, and the separation between plots was always high. Interestingly, one rice field plot was situated between the forest and fallow cluster and the pasture cluster.

Changes within the Different Groups with Land-Use Type

Without exception deforestation had a dramatic effect on the species richness per sample of all taxonomic groups (Fig. 3). Richness was halved in the majority of the groups, with ants, termites, and spiders having the greatest difference between primary forest and rice fields. In old fallows, richness of all groups, except for Coleoptera, was close to that in primary forests. In pastures, richness of most taxonomic groups was very low, in both young plots and old ones. Only the species richness of earthworms was slightly higher in old pastures than in rice fields.

Deforestation had a strong effect on species frequencies for half the groups (Fig. 4). Frequencies of earthworms, diplopods, and chilopods were halved in deforested plots. Deforestation had no effect on frequencies of the other groups. In fallows, species frequencies were close to the primary forest values. Only for earthworms were the frequencies lower in old fallows than in primary forest. In old pastures, however, species frequencies were always lower than in primary forest, with the exception of termites. Moreover, the frequencies of ants, Coleoptera, and spiders were even lower in the old pastures than in the rice fields.

Discussion

Shannon and Simpson Diversity Indexes

The values of the diversity (Shannon) and dominance (Simpson) indexes did not show clear patterns. Both Shannon and Simpson indexes increased in young plots, especially in the young fallow, but were not significantly different between old plots and primary forest plots, whether in pastures or in fallows. Because there was only one young fallow and only two young pastures it was difficult to evaluate the real significance of these hump-shaped curves.

Effect of Forest Clearance on Soil Macrofauna Community

Forest clearance had a strong effect on the soil macrofauna. The community in the rice fields appeared impoverished, with low species richness per sample and per plot. The species richness and frequencies of all groups were affected by deforestation. Moreover, the classification showed that the soil macrofauna communities of the

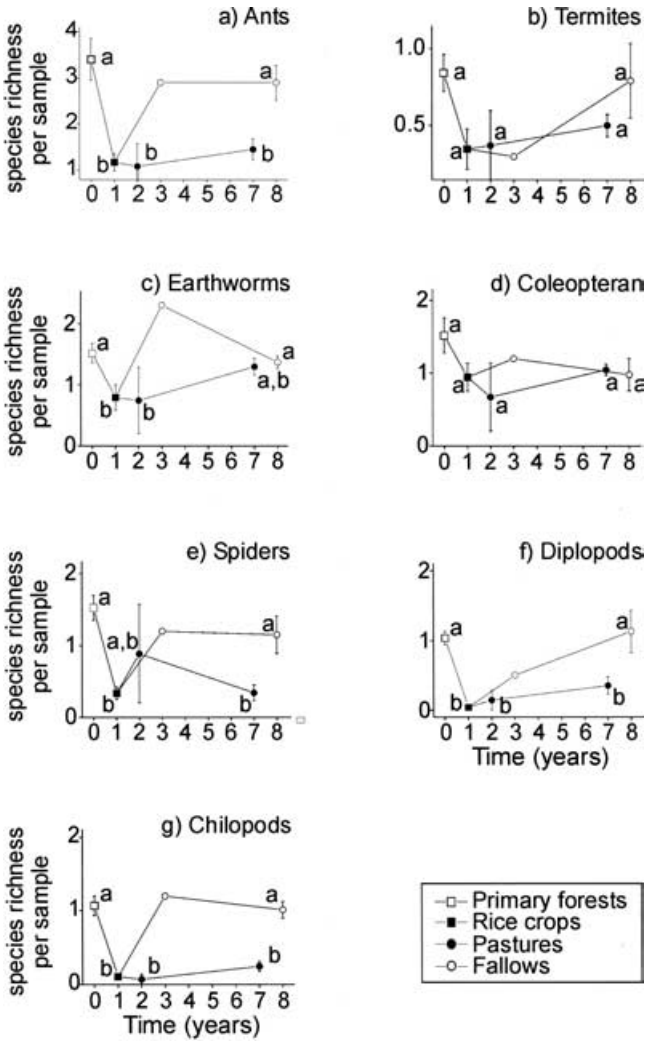

Figure 3. Species richness of the major taxonomic groups of soil macrofauna at the different stages of smallholders farming systems: (a) ants, (b) termites, (c) earthworms, (d) Coleoptera, (e) spiders, (f) diplopods, and (g) chilopods. Stages that do not have common letters are different ($p < 0.05$).

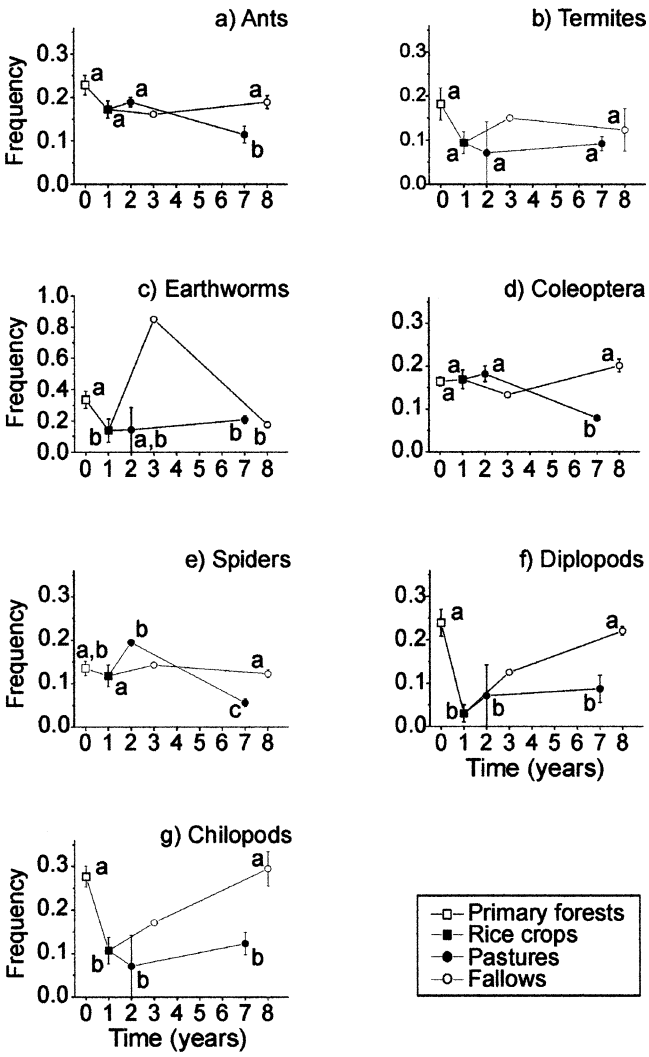

Figure 4. Species frequencies of the major taxonomic groups of soil macrofauna at the different stages of smallholders farming systems: (a) ants, (b) termites, (c) earthworms, (d) Coleoptera, (e) spiders, (f) diplopods, and (g) chilopods. Stages that do not have common letters are different ($p < 0.05$).

rice fields were very different from the communities of the other types of land use. Recently cleared plots of natural vegetation have already been identified as containing depleted soil macrofauna communities (Fragoso et al. 1999). In the Peruvian Amazon the density of soil macrofauna is also much lower in rice fields and pastures than in the primary forest (Lavelle & Pashanasi 1989). In the plots we studied, changes in the soil macrofauna community may have been caused either by the direct effect of fire during the slash-and-burn process or by resulting modification of the vegetation cover.

Results of previous studies do not show a consistent effect of fire on the soil macrofauna. After burning savannas in Colombia, the soil macrofauna community recovered after only 6 months (Decaëns et al. 1994). In Australia the surface arthropods were greatly affected by the first fire but not by subsequent fires (Collet 1998). In a controlled experiment, some species of earthworms benefited from the fire whereas others disappeared (Callham et al. 2003). Ants were affected by fire in a tropical deciduous forest in Mexico (Castano-Meneses & Palcios-Vargas 2003). During a fire, the soil temperature can reach 200° C at a depth of 15 cm (Gimeno-Garcia et al. 2004). Nonmobile invertebrates such as some larvae may not escape from the heat. Very mobile invertebrates, however, such as Coleoptera or spiders, may escape the fire and come back later. In our study, ants, termites, and spiders were the groups most sensitive to deforestation. Ants and termites are protected from fire by their mounds, which are largely subterranean.

Spiders are very mobile organisms and are probably not directly affected by fire.

On the other hand, slash-and-burn fires may have an indirect effect on soil macrofauna by destroying the numerous microhabitats on which many soil invertebrates rely. Decaying wood, fine twigs, dead plant stems, and local accumulations of leaves are sources of food and habitat for numerous species. This loss of plant cover exposes the soil to direct solar radiation, which modifies the climatic conditions of the soil (Strehlow et al. 2002). Most forest-dwelling organisms are adapted to shady and humid environments. In the deforested plots, a large part of this fauna probably was unable to tolerate the shift in microclimatic conditions. For instance, in a Neotropical forest, logging without fire affected the ant community (Castano-Meneses & Palcios-Vargas 2003).

The Soil Macrofauna Community after Rice-Cropping Stage

After the rice-crop stage the soil macrofauna communities were completely different depending on the type of land use. In pastures the communities were impoverished even after 6 years. Moreover, the species became less frequent than in the other stages. Only the termites and the earthworms recovered slightly. The different pasture plots, on the other hand, had a relatively homogenous community, well separated from the communities associated with other types of land use. These results suggest that the soil macrofauna community in pastures developed in a distinctive, homogeneous fashion that is different from the communities associated with the rice fields or fallows. In Amazonia establishing pastures usually leads to an impoverishment of the soil macrofauna community (Fragoso et al. 1999). In Colombia the density of the soil macrofauna is also lower in improved pastures than in the primary forest (Decaëns et al. 1994). In central Amazonia the species richness per sample fell from 156 species in forest to 40 in 15-year-old pastures (Decaëns et al. 2004). Litter invertebrate density was much lower in pastures than in primary forest on Martinique (French West Indies) (Loranger et al. 1999). The ant diversity was halved when pastures were established in another area of central Amazonia (Vasconcelos 1999). In Amazonia the transformation of forest into pasture is also often accompanied by a massive proliferation of earthworms, especially the locally invasive species *Pontoscolex corethrurus* (Lavelle & Pashanasi 1989; Höfer et al. 2001; Barros et al. 2002). We did not, however, find this trend, maybe because the plots were too young.

There are probably numerous causes of degradation in these communities. Change in the environmental conditions, leading to modifications of the soil microclimate and the loss of microhabitat, is certainly an important factor. The remaining microhabitats, such as decaying trunks and grass tufts, are local hotspots of biodiversity in Amazonian pastures (Mathieu et al. 2004). High soil compaction due to trampling by cattle has been reported as a strong limiting factor for the soil macrofauna (Radford et al. 2001). The loss of litter, organic matter (Schroth et al. 2002; Barros et al. 2004), and soil nutrients (McGrath et al. 2001) probably has an effect on the soil macrofauna. The disappearance of some ecosystem engineers such as earthworms (Decaëns et al. 1999; Lavelle et al. 2001) and termites (Jones et al. 1994) that produce biogenic structures used as microhabitats by other species may also accelerate the process of community degradation.

The soil macrofauna community in the fallows showed a very different pattern. It appeared to be a community returning to its initial state after considerable disturbance. The diversity indexes were close to the primary forest values, as were the species frequencies. Most of the groups recovered, especially in terms of diversity. Moreover, the composition of the soil macrofauna of the fallows converged with that of the forest community. The soil macrofauna has a good recovery potential in fallows. For instance, in central Amazonia, the biomass and the diversity of the soil macrofauna in secondary forests are not significantly different from those in the primary forest (Höfer et al. 2001). In a primary forest on Martinique (French West Indies) the litter invertebrate density is higher in fallows than in the primary forest (Loranger et al. 1999). Ants and termites have good recovery potential in fallows. In central Amazonia, the diversity of ants in fallows is close to that in primary forest, although it is halved in pastures (Vasconcelos 1999). In Cameroon, Africa, the composition of the termite community in secondary forests is similar to the composition of the community in primary forest in several studies (Eggleton et al. 1996; Eggleton et al. 2002). This high recovery potential in fallows suggests that fallows are good habitats for the soil macrofauna.

When a plot is abandoned after a rice-crop stage, fast-growing plants such as *Cecropia*, with high litter biomass, grow rapidly (Baar et al. 2000). After 2 or 3 years there is a thick litter layer, and the canopy is already well developed. A microclimate with a low light level and high humidity, similar to a forest microclimate, appears quickly. Moreover, the presence of numerous plant stems and trunks and the thick litter layer provide numerous microhabitats and abundant trophic resources. In the deforested areas of Amazonia, the forest is cleared in patches, creating mosaic landscapes in which deforested plots and primary forest are often close to each other. In such a situation, the soil macrofauna from the remaining forest plots may colonize habitats such as fallows.

Conclusion

Forest clearance was a major disturbance for the soil macrofauna in Amazonia. Immediately after forest clearance, the soil macrofauna community was extremely impoverished and no group seemed to escape this change.

Fallows offered favorable conditions for the soil macrofauna, but the soil fauna in pastures seemed to have a very low recolonization potential. Consequently, in Amazonia, fallows may play an important role in the conservation of soil macrofauna.

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Annexe 4

Choosai, C., J. Mathieu, P. Jouquet, and Y. Hanboonsong, Termite mounds and field bunds as biodiversity refugees in paddy fields in northeastern Thailand. Environmental Conservation, 2009. 36: p. 71-79.

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Termite mounds and dykes are biodiversity refuges in paddy fields in north-eastern Thailand

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SUMMARY

Paddy fields in north-eastern Thailand are heterogeneous agro-ecosystems that can be described as mosaics of paddy rice plots, dykes and termite mounds. The aim of this study was to determine if this heterogeneity influences soil macrofauna biodiversity. While biodiversity did not vary as a result of different rice management practices (direct seeding and transplanting), dykes and mounds were vital to the maintenance of soil macrofauna biodiversity. Diversity and density were higher in termite mounds and field dykes, compared to rice plots, especially during the rainy season. Consequently, termite mounds and dykes can be considered to be biodiversity hotspots that behave as refuges for other soil macrofauna during the rainy and dry seasons, providing protection against flooding and dryness. The importance of these patches of biological activity in terms of ecosystem functioning and services are discussed.

Keywords: biodiversity, heterogeneity, paddy field, soil macrofauna, termite mound, Thailand

INTRODUCTION

The search for self-sustaining, low input, diversified and energy efficient agricultural systems is currently of major concern to researchers, farmers and policy makers worldwide (Foley *et al.* 2005). Maintaining biodiversity is one of the key targets of sustainable agriculture because of its increasingly recognized positive effects on nutrient cycling, pest population regulation and plant growth (Matson *et al.* 1997; Mäder *et al.* 2002). Biodiversity also offers potentially important sources of food and medicine, and even plays a valuable part in myth and folklore (Altieri 1995).

In north-eastern Thailand, 35% of the landscape is occupied by paddy fields (Tomita *et al.* 2003), which are very constraining environments for the development of soil macrofauna. Soil macrofauna activity is limited during the rainy season by the anoxic conditions caused by flooding and then in the dry season by the very dry weather. Paddy fields are heterogeneous ecosystems owing to the presence of many small plots separated by small elevated embankments made of soil, called 'dykes' (with an average height of 40cm), which are generally covered by many types of grasses. Another striking feature of these ecosystems is the presence of mounds created by termites, on which various kinds of trees, shrubs and sometimes grasses grow all year round. These two sources of heterogeneity may be important for soil biodiversity preservation, providing refuges for soil macrofauna during the rainy season while paddy fields are flooded, and offering the shade and humidity necessary for their development and survival during the dry season.

As ecosystem engineers (*sensu* Jones *et al.* 1994, 1997; Lavelle 1997; Jouquet *et al.* 2006, 2007), termites play a prominent role in maintaining biodiversity. These soil-dwelling organisms modify soil properties by displacing soil organic and mineral compounds from one site to another and by producing biogenic structures, namely organo–mineral aggregates (faeces, mounds, aggregates and gallery walls) and macropores (galleries, chambers), with specific physical, chemical and biological properties (de Bruyn & Conacher 1990; Black & Okwakol 1997; Holt & Lepage 2000; Jouquet *et al.* 2006). Soil ecologists usually consider these structures as activity hotspots and high resource patches, sometimes referred to as fertility 'islands' (Smith & Yeaton 1998; Konaté *et al.* 1999; Jouquet *et al.* 2006, 2007), which create spatial variability in soil properties at the ecosystem scale (Schuurman 2006; Obi & Ogunkunle 2009).

In this study, we assessed the role of dykes and termite mounds in sheltering soil macrofauna biodiversity in paddy fields. We compared biodiversity in rice plots to that in dykes and termite mounds in fields managed following the two most common management practices used for rice cropping in this region: direct seeding and transplanting. We also examined whether patterns of biodiversity distribution were similar during the dry and rainy seasons.

METHODS

Study sites

The study was conducted in paddy fields (rice crops) in north-eastern Thailand (Khon Kaen province, Ban Fang amphur, Baan Daeng village, 102.62°E and 16.38°N). This area is largely dominated by steep hills with slopes of up to 200 m altitude. In the past, the area was forested and rice growing

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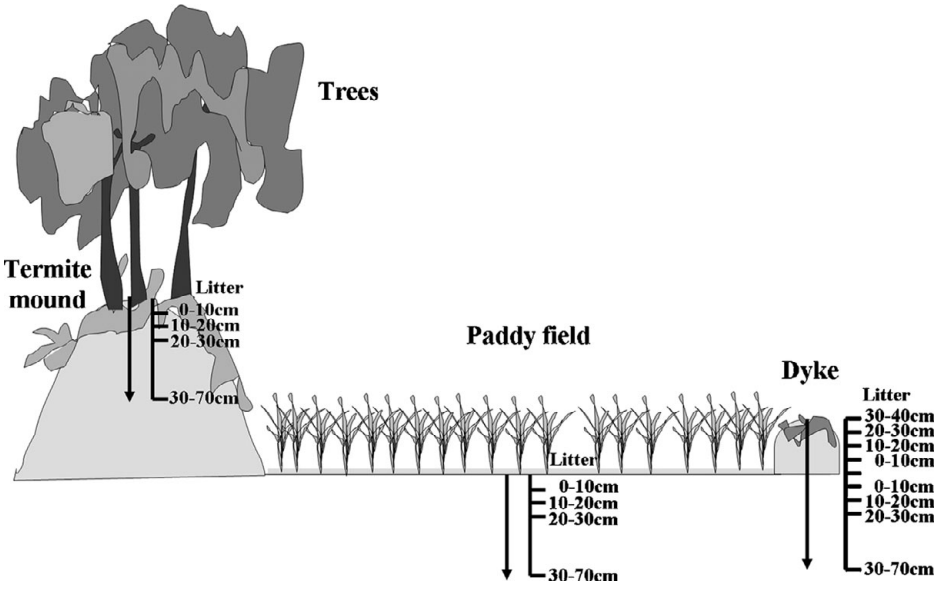

Figure 1 Soil macrofauna was sampled to a depth of 70 cm in dykes, paddy fields and termite mounds, and to a height of 40 cm in dykes ($n = 6$ replicates of five modified tropical soil biology and fertility samples at each location in each season).

started 25–35 years ago. The soils are typical Natraqualf (Soil Survey Division Staff 1998) from the Kula Ronghai Thailand soil series. These paddy soils are very compact with a bulk density of 1.78 and 2.01 mg cm^{-3}, in the Ap and Bt horizon, respectively. The pH is slightly acidic (≈ 6) in the topsoil and neutral in the subsoil. The clay fraction is mostly kaolinitic with some smectites (Saejiew *et al.* 2004).

The area has a humid tropical climate with distinct rainy and dry seasons. Annual rainfall is *c.* 1000 mm, with 90% of rainfall occurring between May and October. In our sample year (2007), the annual rainfall was *c.* 1300 mm. During the rainy season (June–November) the temperature is 25–33 °C, with 82% mean humidity. During the dry season temperature is 16–30 °C (mean humidity 70%).

In this region, rice plots are managed using two dominant practices: direct seeding (hereafter called 'DS plots') and transplanting (hereafter called 'TP plots'). In TP plots, seedlings are transplanted from a nursery to the field, whereas in DS plots, rice seeds are sown manually in the field. In both cases, plots are small (0.1–0.2 ha) and are separated by approximately 40 cm high and 40 cm wide soil embankments, called 'dykes'. During the rainy season, many kinds of shrubs and grasses grow naturally on these dykes and also in the plots. Almost all the paddy fields are flooded (for a duration of 1–4 months), and the water is retained by the dykes. At the beginning of the season, the water is usually 30 cm deep and then evaporates, becoming shallower towards the end of the rainy season. After rice harvesting, the field is left fallow during the dry season, and soils become very dry, except in mounds and dykes that are still covered by vegetation.

Termite mounds are widespread in the study region, with approximately 2 mounds ha^{-1}, and occur only at the junction of dykes, at the corner of plots. They can reach 2 m in height and 4 m wide and are always covered by many types of trees, such as Siamese rough bushes *Streblue asper* and the neem tree *Azadirachta indica* Adrien de Jussieu var. *siamensis*.

Sampling design

The soil macrofauna was sampled in five types of locations (Fig. 1): (1) termite mounds; (2) inside the rice TP plots; (3) inside the rice DS plots; (4) in the dykes between two TP plots (hereafter called 'TP dykes'); and (5) in the dykes between two DS plots (hereafter called 'DS dykes'). We sampled soils in both the rainy and dry seasons (August 2007 and February 2008) with $n = 6$ replicates for each location. Replicates were randomly selected from the landscape and were at least 150 m apart. During the rainy season, sampling was done when water reached 5 cm depth in average. Each replicate consisted of the addition of five modified tropical soil biology and fertility (TSBF) samples (Anderson & Ingram 1993) randomly located within each location type (composite samples).

Soil macrofauna sampling

Following the standard TSBF method (Anderson & Ingram 1993), we manually removed soil sample blocks 25 cm wide × 25 cm wide × 10 cm depth. We modified this method by increasing the depth of the blocks to 70 cm, which gave us five successive strata: litter, 0–10 cm, 10–20 cm, 20–30 cm and 30–70 cm below ground. We included an extra four layers when sampling the dykes: 0–10 cm, 10–20 cm, 20–30 cm and 30–40 cm above ground (Fig. 1). Soil macro-invertebrates (>2 mm in size) were removed from each layer of soil by hand-sorting. Individuals were preserved in 70% alcohol, except for earthworms, which were preserved in 4% formalin solution for two days and then transferred back to 70% alcohol. Soil macro-invertebrates were counted and classified into taxonomic groups and identified at the morpho-species level (Oliver & Beattie 1993; Oliver & Beattie 1996). Those species which play a significant role as rice pest predators or which are occasionally eaten by farmers were also identified at the species level.

Data analysis

The macrofauna data were log(x + 1) transformed when necessary and analyses of variance (ANOVA) were performed. Means were compared by Tukey tests. Differences in species compositions and community structure were assessed by principal component analysis (PCA) on the abundance of each group and by comparing the species or broad taxonomic groups in common between the locations. Species richness was defined as the total number of morpho-species. The diversity was described by species richness (R), the Shannon (H') index and Shannon evenness (H'/ln(R)). Abundance was defined as the number of individuals per m^2. Species specific to particular locations were identified using the indicator value (Indval) method (Dufrene & Legendre 1997), which combines the frequency and abundance of the species. To use this method, locations were classified according to the PCA outcomes. All statistical analyses were performed with R (R Development Core Team 2008), in particular using the Coan package for community analyses (URL http://www.jerome.mathieu.freesurf.fr/coan_engl.htm).

Table 1 Diversity indices (species richness R, Shannon index H' and Shannon evenness H'/ln (R)) of the soil macrofauna for each location and season (DS = direct seeding, TP = transplanting).

Sample location	*Dry*	*Rainy*	*Overall*
Species richness (R)			
Mound	58	52	80
Dyke-DS	35	43	55
Dyke-TP	32	44	57
Plot-DS	30	30	49
Plot-TP	32	15	40
Shannon index (H')			
Mound	2.66	2.10	2.41
Dyke-DS	1.43	1.66	1.67
Dyke-TP	1.85	1.71	1.93
Plot-DS	1.92	1.84	2.10
Plot-TP	2.30	1.45	2.11
Shannon evenness H'/ln (R)			
Mound	0.65	0.53	0.55
Dyke-DS	0.40	0.44	0.42
Dyke-TP	0.54	0.45	0.48
Plot-DS	0.56	0.54	0.54
Plot-TP	0.66	0.53	0.57

RESULTS

Biodiversity

A total of 118 macrofauna morpho-species was found, distributed among 41 families and 14 orders. Eight taxonomic groups were commonly found: earthworms, termites, ants, spiders, coleopterans, orthopterans, chilopods and diplopods.

Biodiversity was highest in termite mounds regardless of the parameter considered, whereas it was always lowest in plots. The total species richness was nearly twice as high in termite mounds (80 species) than in the plots (40 and 49 in TP and DS, respectively) (Table 1). Total species richness was intermediate in the dykes (57 and 55 in TP and DS, respectively). Average species richness followed the same trend, with 22 species in termite mounds in the dry and rainy seasons compared with 15 in other locations during the dry season, and then 15 in dykes and six in plots in the rainy season (Fig. 2). This trend was observed for most groups, but especially for ants, termites and spiders. However, orthopteran distribution differed, with equal species richness in each location in the dry season and highest species richness in DS dykes. Myriapod species richness did not vary between location and season. In summary, during the rainy season, species richness increased in dykes while it decreased inside plots. Conversely, species richness remained the same in termite mounds in both seasons.

The diversity, as measured by the Shannon index, varied with location and season (Table 1). It was maximal in the mounds in both seasons (2.66 and 2.10 for dry and rainy seasons, respectively) and minimum in DS dykes (1.43) in the dry season and in TP plots (1.45) in the rainy season. Overall, diversity was lower during the rainy season than during the dry season, except in DS dykes, where conversely diversity was higher during the rainy season.

The diversity, as measured by the Shannon evenness, varied with location and season (Table 1). It was maximal in TP plots (0.66) in the dry season and in DS plots (0.54) in the rainy season, while it was minimal in DS dykes in both seasons (0.40 and 0.44 for dry and rainy seasons, respectively). Overall, diversity was lower during the rainy season than during the dry season, except in DS dykes.

Density

The overall soil faunal density showed the same pattern as species richness. It was higher in termite mounds than in other locations, especially during the rainy season (Fig. 3). The total density increased in dykes during the rainy season but it did not change inside the plots. The density of termites, coleopterans, spiders and myriapods followed the same pattern as total density, with higher density in the rainy season and in termite mounds. Conversely, ants, earthworms and orthopterans were found at a higher density in dykes than in mounds, especially during the rainy season. Overall, the density of soil macrofauna decreased with increasing soil depth, except in termite mounds where density increased with depth (Fig. 4).

Community structure

The PCA clearly isolated three clusters: termite mounds, dykes and plots (Fig. 5). Samples were not grouped according to land use (DS or TP) suggesting that it did not have a significant effect on community structure. The correlation circle indicated that a high density of termites, spiders,

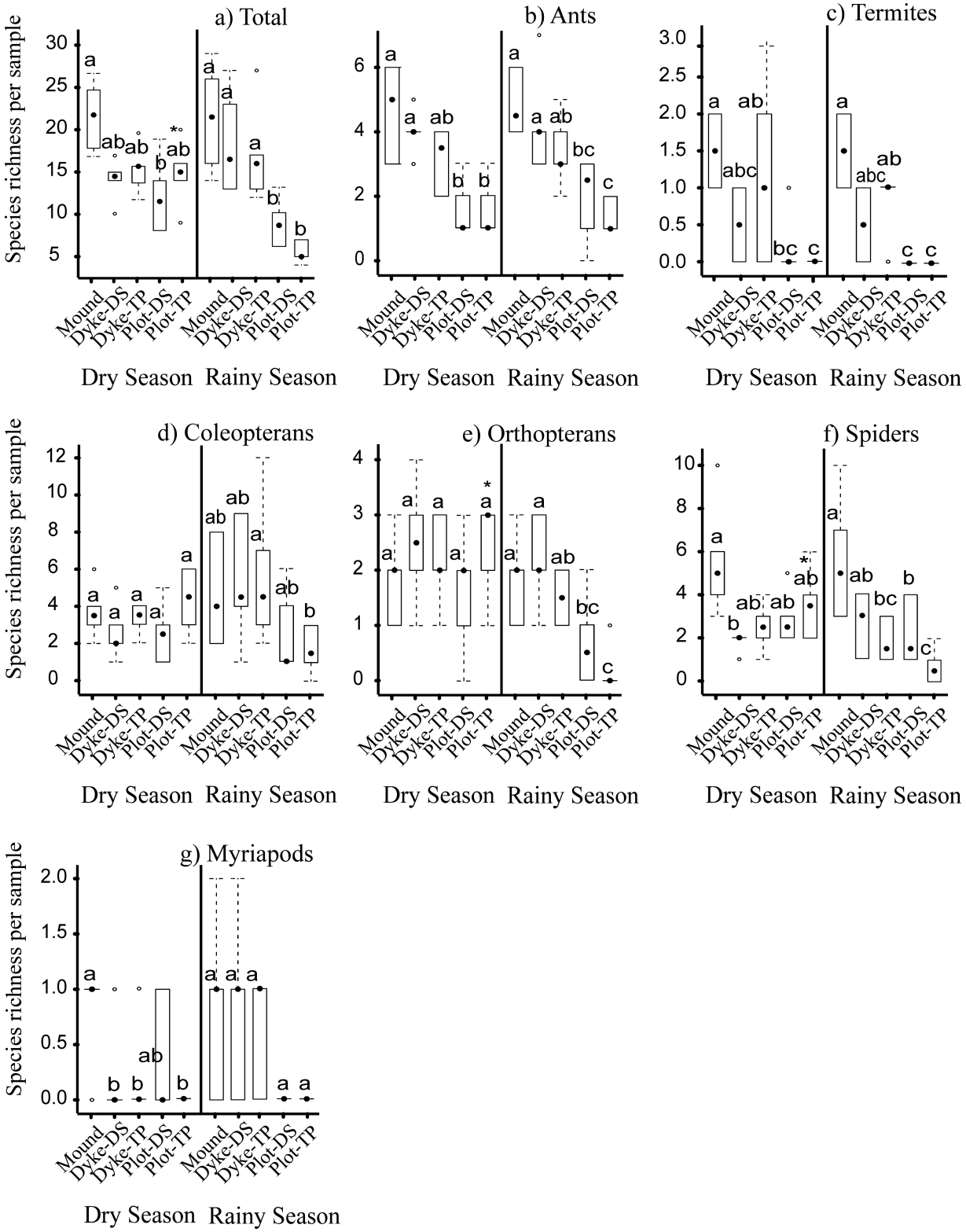

Figure 2 Box and whisker plots of average species richness of the different soil macrofauna groups per location and season. (*a*) Total soil macrofauna, (*b*) ants, (*c*) termites, (*d*) coleopterans, (*e*) orthopterans, (*f*) spiders and (*g*) myrapods. DS = direct seeding technique, TP = transplanting technique. Histograms with the same letters are not significantly different at $p = 0.05$, $n = 6$.

chilopods and diplopods characterized the termite mound cluster, whereas orthopterans, earthworms and ants were the main characteristic features of dykes (Fig. 5*b*). Our previous results concerning species density also identified these groups of fauna due to their similar habitat preferences. Paddy plots were characterized by a low density of all groups.

The three clusters determined by the PCA showed little resemblance in species composition. Dykes and plots were

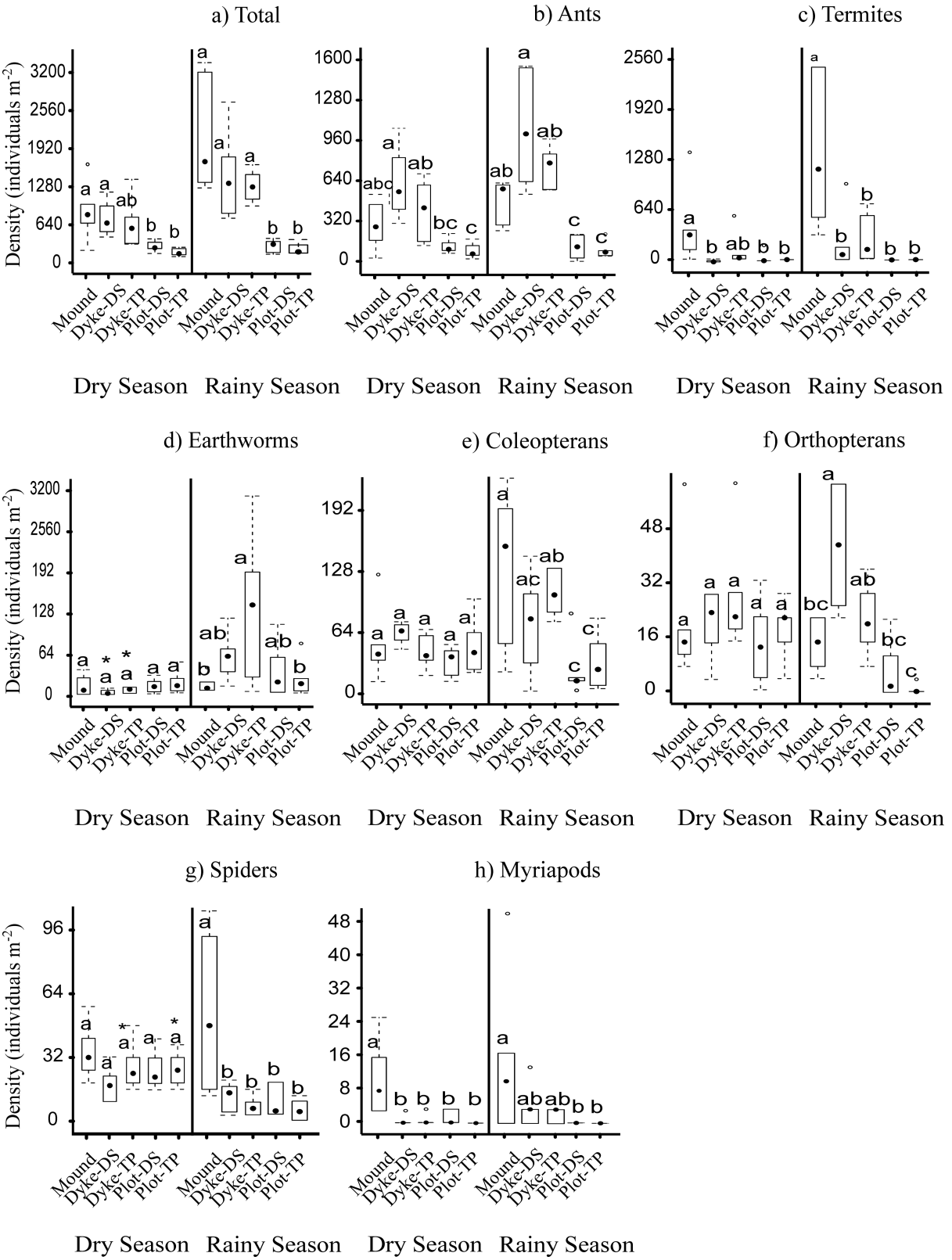

Figure 3 Box and whisker plots of mean density of the different soil macrofauna groups per location and season. (*a*) Total soil macrofauna, (*b*) ants, (*c*) termites, (*d*) earthworms, (*e*) coleopterans, (*f*) orthopterans, (*g*) spiders and (*h*) myrapods. DS = direct seeding technique, TP = transplanting technique. Histograms with the same letters are not significantly different at $p = 0.05$, $n = 6$.

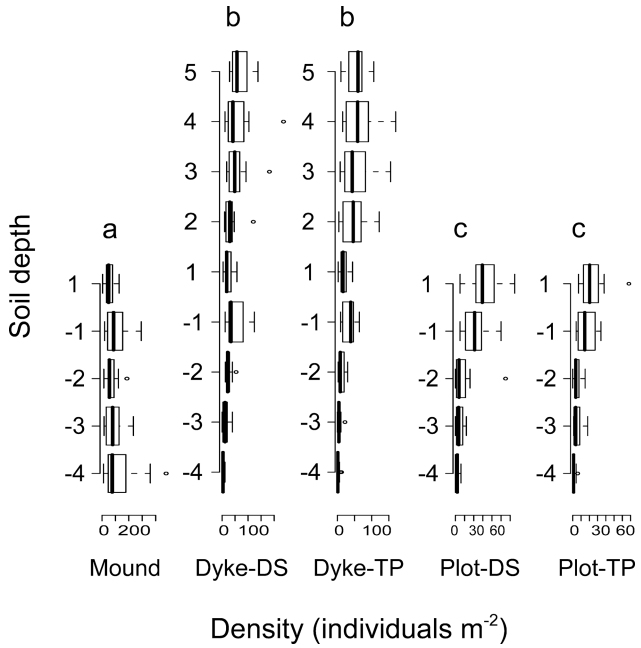

Figure 4 Box and whisker plots of mean density of the soil macrofauna per location and depth. DS = direct seeding technique, TP = transplanting technique, L = litter, −1 to −4 = 0–10 cm, 10–20 cm, 20–30 cm and 30–70 cm below ground, 1 to 4 = 0–10 cm, 10–20 cm, 20–30 cm and 30–40 cm above ground. Histograms with the same letters are not significantly different at $p = 0.05$, $n = 6$.

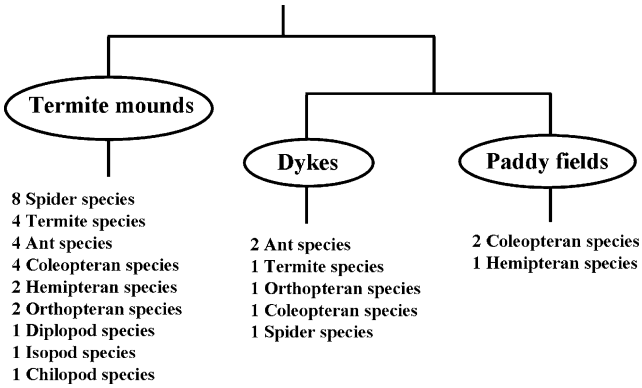

Figure 6 The number of specialist species (according to Indval scores) in the clusters identified by principal component analysis (see Fig. 5).

the most similar, with 56% species in common, mounds and dykes were the most different, with only 38% of species in common. Forty-two per cent of the species were found both in mounds and plots (Fig. 5*a*).

Indicator species

Among the 118 morpho-species observed in the different locations, 36 (30.5%) were significant indicators of a PCA cluster (Fig. 6) according to their Indval values: 27 mound specialist species were found including four species of soil and litter feeder termites (*Odontotermes formosanus*, *Hospitalitermes ataramensis*, *Angulitermes* sp. and *Microcerotermes* sp.), four species of ants (omnivores and predators), eight species of spiders (predators), four coleopteran species (two omnivorous and two predators), two species of hemipterans (omnivorous), two orthopterans (Blattellidae: detritivore and Phasmatidae: omnivorous), and one species of chilopod (predator). Mounds were the only habitat of specialist detritivores such as millipedes (one species) and isopod (one species). Six specialist species inhabited dykes: two ant species (omnivorous and predators), one species of spider (predator), one soil feeder termite species (*Pericapritermes* sp.), one orthopteran species (Gryllotalpidae, grass feeder) and one species of coleopteran (Scarabaeidae, omnivorous). Rice plots provided a habitat for only three specialist species: two coleopterans (Carabidae, predators) and one species of hemipteran (omnivorous).

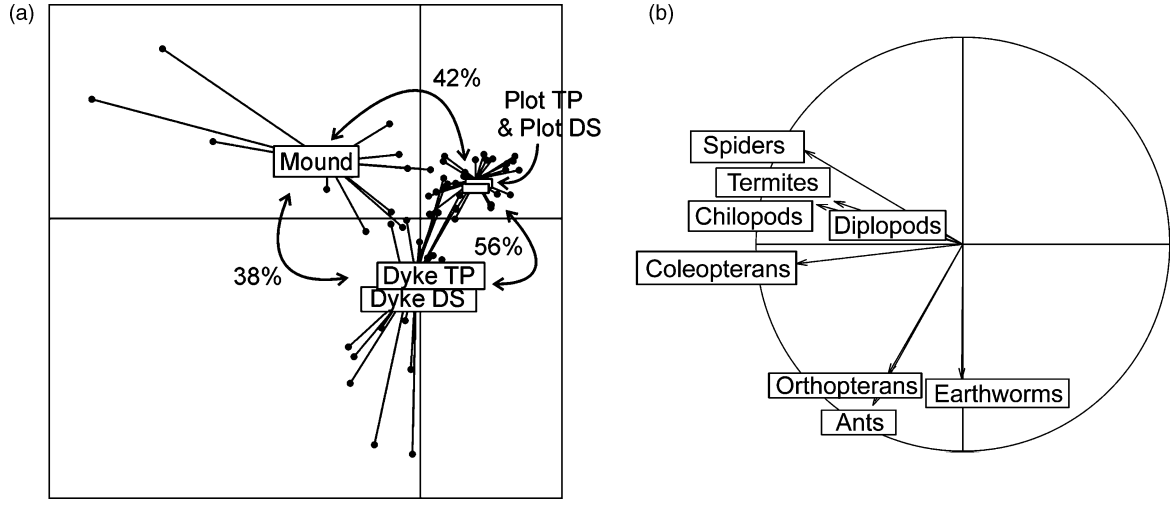

Figure 5 Principal component analysis performed on the density of macrofauna groups. (*a*) Projection of the samples on axes 1 and 2 of the PCA. Arrows indicate the percentage of species in common between the three clusters. (*b*) Correlation circle between the variables. DS = direct seeding, TP = transplanting technique.

DISCUSSION

Land management practices and biodiversity in paddy fields

Both direct seeding and transplanting practices are used in north-eastern Thailand. These practices differ in several aspects which generate different habitat conditions that might affect the soil macrofauna. Firstly, transplanting practices require flooding while direct seeding can be done without it (Miyagawa *et al.* 1998). The shorter flooding period in direct seeding fields (almost a month less) can be assumed, at first glance, to be more favourable for soil macrofauna. Secondly, although these two systems do not show significant differences in soil properties such as soil organic matter content and pH (Clermont-Dauphin *et al.* 2005) and weed abundance (Tomita *et al.* 2003), weed species-richness is higher in direct seeding fields than in transplanted ones. Hence, food diversity is higher in direct seeding plots. Therefore direct seeding may provide a more favourable environment for soil macrofauna because of reduced flooding time and higher food diversity. However, we found no significant difference in soil macrofauna density and species richness between the two systems. Because of the low density and diversity in rice fields regardless of planting regimes, it appears that soil macrofauna has difficulty surviving in these environments. This scarcity and low level of diversity may be explained by the harshness of rice crops, which are flooded for 1–4 months of the year and severely dry for 5–7 months, as well as having low levels of food resources (low litter and soil organic matter contents). Ploughing before rice planting and after rice harvesting, which was reported to severely affect soil macrofauna (Lavelle & Pashanasi 1989; Wardle *et al.* 1993), may have also contributed to lowering biodiversity levels in paddy fields.

The soil macrofauna was sampled in August, after flooding the paddy fields in June and July and before soil was expected to become totally dry in appearance. Surprisingly, spiders and ants were found on rice leaves and soil surfaces in areas that were partially covered by water, demonstrating that some soil macrofauna groups can easily colonize surrounding areas from the dykes. In addition, sampling showed that soil macrofauna can survive when the soil is flooded. Even when the paddy field was flooded on the surface and the soil moisture content was high, it was not saturated at depths of 0–30 cm. We therefore believe that the soil surface is acting as a crust, lowering water diffusion deep into the soil and impeding oxygen outflow, thus allowing soil macrofauna to survive.

Termite mounds and dykes are biodiversity hotspots

Soil macrofauna communities were strongly influenced by the season and the local environmental and habitat conditions in paddy fields. The density and species richness of soil macrofauna were higher in the rainy than in the dry season (except in the case of the plots). Since biodiversity was higher in termite mounds (greater species-richness and specificity), intermediate in dykes and the lowest in the rice plots, this ecosystem can be considered as a mosaic with two discrete hotspots: mounds and dykes surrounded by a matrix of rice plots with low soil macrofauna species-richness.

Five different termite species were found in the termite mounds (*Odontotermes formosanus*, *Hospitalitermes ataramensis*, *Macrotermes gilvus*, *Angulitermes* sp. and *Microcerotermes* sp.). *M. gilvus* was originally suspected to have constructed the termite mounds (Sawaeng Ruaysoongnern, personal observation 1988). However, *M. gilvus* was only found in two mounds, whereas *O. formosanus* was found in all mounds and was the most dominant termite species in every case. We therefore hypothesize that the termite mounds were generated by the activities of different termite species, and that *O. formosanus* became the main species involved in mound edification and dynamics after *Macrotermes* sp. colonies died. A similar mechanism of termite mound dynamics was observed in African savannah ecosystems (Konaté 1998).

Termite mounds create islands of fertility for grasses, trees and animals (Holt & Lepage 2000; Fleming & Loveridge 2003; Jouquet *et al.* 2004, 2006; Diehl *et al.* 2005; Mwabvu 2005; Scott *et al.* 2006). Hence, increased biodiversity within termite mounds might be explained by the better living-environment for soil and litter-inhabiting macrofauna, namely higher substrate levels (litter and soil organic matter), better protection from direct sunshine and more favourable soil moisture conditions. Shadow and litter from trees may be especially important for litter-inhabiting macrofauna (such as spiders and orthopterans) which are prone to desiccation (Hofer *et al.* 2001) and which could not survive in the surrounding dry environment. During the rainy season, soil macrofauna that need to live in flood-free systems can survive in the mounds and dykes. Concurrently, during the dry season mounds provide an environment with sufficient moisture content for soil macrofauna to survive. Although we found that density increased with depth in termite mounds, our samples were only taken down to a depth of 70 cm, which means that the actual biodiversity within mounds could be significantly higher than that found in our study. Therefore our sampling procedure probably underestimated the positive effect of termite mounds on soil macrofauna biodiversity.

Biodiversity and ecosystem services in paddy fields

This study stresses the importance of ecosystem engineering activity (such as human activity due to dyke construction and natural activity by termites building the mounds) in the maintenance of spatial heterogeneity in paddy fields and with implication for soil macrofauna biodiversity conservation. Few studies have examined the impact of soil macrofauna in the functioning of partially flooded ecosystems such as paddy fields, although ecosystem functions and services may be influenced by its biodiversity. Jouquet *et al.* (2008) previously reported the possible effect of ants and earthworms on soil particle size and soil organic matter dynamics in paddy fields in the same study area. In Indonesia, Widyastuti (2002) also demonstrated that soil macrofauna plays an important

role in promoting litter decomposition and mineral nitrogen dynamics. Soil biodiversity might also be important regarding the role of soil macrofauna as pests or predators of rice pests. Ant and spider densities were found to be higher on/in mounds and dykes. These two patches therefore constitute refuges where they can survive and from which they could colonize paddy fields. Ants and spiders are efficient predators and can act as agents in the control of rice pests (Settle *et al.* 1996). Although we did not find any soil macrofauna pests in our study, termite mounds and dykes provide a haven for soil macrofauna predators to shelter and could thus constitute a sustainable resource for controlling rice pests. Finally, insects are consumed as food by people in many parts of the world, including north-east Thailand (Borror *et al.* 1992), and a survey revealed that some of the soil macrofauna species found in our study were eaten by local inhabitants (Chutinan Choosai, unpublished data 2008). Amongst the sampled soil macrofauna species, two species are occasionally consumed: one ant species (Formicidae: *Oecophylla smaragdina*), which was only found in the mounds, and one orthopteran species (*Gryllotalpa africana*), mainly found in the dykes during the rainy season (19.7 individuals m^{-2} in the dykes, 2.1 individuals m^{-2} in the paddy field plots). Conserving dykes and termite mounds could therefore constitute a significant dietary supplement for local farmers.

In conclusion, paddy fields in north-eastern Thailand are adverse environments for soil macrofauna. In these agricultural landscapes, mounds and dykes can be considered to be local biodiversity hotspots, providing shelter for many soil macrofauna species. Since these species are involved in ecosystem functions and services, their conservation should be integrated into sustainable rice management systems.

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Annexe 5

Dupont L., Grésille Y., Richard B., Decaëns T. and Mathieu J. Fine-scale spatial genetic structure and dispersal constraints in two earthworm species. Biological Journal of the Linnean Society, in press

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Dispersal constraints and fine-scale spatial genetic structure in two earthworm species

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The limited dispersal ability of earthworms is expected to result in marked genetic isolation by distance and remarkable spatial patterns of genetic variation. To test this hypothesis, we investigated, using microsatellite loci, the spatial genetic structure of two earthworm species, *Allolobophora chlorotica* and *Aporrectodea icterica*, in two plots of less than 1 ha where a total of 282 individuals were collected. We used spatial autocorrelation statistics, partial Mantel tests of isolation-by-distance (IBD) and isolation-by-resistance (IBR), and Bayesian test of clustering to explore recent patterns involved in the observed genetic structure. For *A. icterica*, a low signal of genetic structure was detected, which may be explained by an important dispersal capacity and/or by the low polymorphism of the microsatellite loci. For *A. chlorotica*, a weak, but significant, pattern of IBD associated with positive autocorrelation was observed in one of the plots. In the other plot, which had been recently ploughed, two genetically differentiated clusters were identified. These results suggest a spatial neighbourhood structure in *A. chlorotica,* with neighbour individuals that tend to be more genetically similar to one another, and also highlight that habitat perturbation as a result of human activities may deeply alter the genetic structure of earthworm species, even at a very small scale. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **114**, 335–347.

ADDITIONAL KEYWORDS: gene dispersal – genetic clustering – genetic diversity – isolation by distance – soil biodiversity – soil properties.

INTRODUCTION

Dispersal, which is the main mechanism leading to gene flow within and between populations, directly influences the level of genetic diversity maintained in populations (Clobert, 2001) and the ability of species to expand their range (Holt, 2003). The dispersal capacity of species is thus a fundamental life-history trait that plays a central role in the evolution of populations and their spatio-temporal dynamics. Limited dispersal ability can lead to mating among related individuals and should thus result in marked genetic isolation by distance and remarkable spatial patterns of genetic variation (Arnaud *et al*., 2001).

Soil invertebrates, such as earthworms, which are known to contribute to the maintenance of soil structure, the regulation of soil organic-matter dynamics,

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and the stimulation of soil fertility and plant growth (Edwards, 2004), are believed to have restricted dispersal abilities (Costa *et al*., 2013) and to have developed original dispersal strategies as a result of the solidity, opacity, and high spatio-temporal heterogeneity of the soil environment (e.g. Mathieu *et al*., 2010). Determining the scale at which gene flow occurs in the field could indicate the approximate scale of demographic independence of earthworm populations and may inform on connectivity of vulnerable populations (Wilson *et al*., 2011). In a context of agricultural intensification (e.g. fertilization, pesticide application, and tillage), which results in a decline of soil biodiversity (Liiri *et al*., 2012), understanding dispersal patterns is crucial for the management of earthworm populations.

Some observations of introduction into earthworm-free habitats suggest that earthworms are able to colonize new areas at distances ranging from 4 to 14 m $year^{-1}$ (review in Mathieu *et al*., 2010; Eijsackers, 2011). Mesocosm experiments have also indicated that some species can travel longer distances (26–500 m $year^{-1}$) under conditions that trigger dispersal (Mathieu *et al*., 2010; Caro *et al*., 2013). Moreover it has been suggested that passive dispersal, either by anthropogenic or natural processes (i.e. surface run-off or phoretic factors), should result in long-distance dispersal (Marinissen & Vandenbosch, 1992, Eijsackers, 2011). Earthworm dispersal has rarely been indirectly estimated using genetic data, and scarce data are available on the level of gene flow within and between earthworm populations. The studies carried out to date have focused on standard techniques, such as estimation of F_{ST} (i.e. proportion of variance in allele frequencies that is among populations) and related statistics to measure gene flow. Most authors who have tested the relationship between genetic and geographical distances between earthworm populations using these population-based measures, whereby species are arbitrarily divided into partially isolated populations, have found significant genetic differentiation and no or a weak isolation-by-distance (IBD) pattern (Enckell *et al*., 1986; Kautenburger, 2006; Cameron, Bayne & Coltman, 2008; Prasankok *et al*., 2013; Torres-Leguizamon *et al*., 2014, but see Novo, Almodóvar & Díaz-Cosín, 2009; Novo *et al*., 2010a). All these studies were carried out at the regional/landscape scale, except that of Novo *et al*. (2010a), which investigated the genetic structure of a homogastrid earthworm at fine spatial scale using *F*-statistics (0.064 km^2). Such unpredictable genetic-differentiation patterns were interpreted as evidence of the key role of anthropogenic or animal-mediated transport for earthworms' dispersal (Costa *et al*., 2013). Whilst the IBD model is based on the assumptions that populations are large, equal in size, and stable over time, computer simulations have shown that unpredictable patterns of IBD are obtained when allowing for different population sizes and random fluctuations of population size and when adding environmental noise (Bjorklund *et al*., 2010). Interpreting an IBD pattern, or a lack thereof, is thus a hard task when studying populations probably subjected to perturbation and fragmentation, such as earthworm populations in agricultural areas. In contrast to population-based approaches, individual-based methods, such as spatial autocorrelation analysis (Hardy & Vekemans, 1999), directly analyse the genotypes of individuals across space, and thus estimates of population structure are not affected by a priori delimitation of populations. These estimates may then be used to infer the biological processes leading to clustering of genotypes (e.g. Carriconde *et al*., 2008).

Here, we aimed at obtaining a better understanding of gene flow in earthworms at fine spatial scale (within plots measuring less than 1 ha) by analysing patterns of genetic structure using individual-based approaches in addition to traditional population-based measures. Moreover, we tested whether gene flow between individuals was constrained or facilitated by soil properties [isolation by resistance (IBR)], which were described using soil resistivity as a single synthetic variable. Resistivity represents the capacity of the soil to resist the flow of electricity, which is tightly linked to soil physical and chemical properties, such as texture, hydrological properties, or nitrogen content, and can be measured almost continuously in space, offering a much better picture of spatial variations of soil properties than do traditional soil analyses. In several studies, the abundance of endogeic and anecic earthworms was highly related to soil resistivity (Valckx *et al*., 2009; Joschko *et al*., 2010; Lardo *et al*., 2012). However, the correlation was positive or negative depending on the species (Valckx *et al*., 2009).

In this study, we focused on two endogeic (i.e. species living in the upper organo-mineral soil layers and forming horizontal nonpermanent burrows, Bouché, 1977) earthworm species commonly found in European agricultural soils, the green morph of *Allolobophora chlorotica* (Savigny, 1826) and *Aporrectodea icterica* (Savigny, 1826). *Allolobophora chlorotica* is known to be located in the upper 60-mm soil layer (Sims & Gerard, 1999), is theoretically able to travel more than 167 m $year^{-1}$ in constant suitable conditions, and is not subject to density-dependent dispersal (Caro *et al*., 2013). *Aporrectodea icterica* is found deeper in the soil and is considered to be more mobile, being able to travel up to 500 m $year^{-1}$ under constant artificial conditions

and to respond to density (Mathieu *et al*., 2010; Caro *et al*., 2013).

By comparing these two earthworm species belonging to the same eco-morphological group but with contrasted dispersal capabilities, we tested the hypothesis that restricted-disperser species should present a higher degree of spatial organization than high-disperser species. Specifically, the objectives of this study were: (1) to estimate the total genetic diversity of these earthworm species in plots of less than 1 ha; (2) to determine the spatial genetic structure and scale of IBD and IBR in these plots; and (3) to estimate gene dispersal within plots if IBD is found.

MATERIAL AND METHODS

Biological models

Aporrectodea icterica is an abundant diploid and obligatory biparental earthworm species (Casellato, 1987) that is commonly found in agricultural soils (Capowiez *et al*., 2005). Its taxonomic status is firmly grounded and the species has distinct morphology, making it easy to recognize (Torres-Leguizamon *et al*., 2012). Conversely, the *A. chlorotica* aggregate (Dupont *et al*., 2011) is composed of several sister species. A green colour morph of this aggregate represents a single taxon although composed of two mitochondrial lineages, whilst the taxonomic status of a pink morph (at least five mitochondrial lineages) remains unclear (King, Tibble & Symondson, 2008; Dupont *et al*., 2011). Although hybridization seems possible between morphs (Dupont *et al*., 2011), introgression is probably restricted as a result of postzygotic reproductive isolation. Cross experiments indeed revealed: (1) a severely restricted viability of cocoons produced by the green morph in pink–green pairings; and (2) male sterility of the surviving hybrids (Lowe & Butt, 2008). Here, we restricted our study to the species represented by the green morph. This diploid and amphimictic species is common in temperate grassland (Lowe & Butt, 2007).

Study site and soil properties

The sampling was carried out at the 'Lycée Agricole d'Yvetot' (Seine Maritime, France), located 200 km north-west of Paris, during March and April 2009. We selected two pastures, ~500 m apart, located in the same topographic situation but with contrasting ages: a 5-year-old pasture (P_A) and a pasture of more than 42 years of age (P_B). In each pasture, sampling was carried out on a 10-m mesh grid of 120 m × 70 m; 104 points per plot were sampled for P_A but, for logistical reasons, the number of points was reduced to 68 in P_B (latitude/longitude range in Lambert II étendu: 484496–484634/2513651–2513774 and 484058–484197/2513760–2513882 in P_A and P_B, respectively).

Soil resistivity was measured in partnership with Geocarta (Geocarta SA, Paris). The automatic resistivity profiling (ARP) (Papadopoulos *et al*., 2009) technique was chosen for its high accuracy and its reduced sensitivity to superficial geophysical noise. Moreover, this technique is non-invasive and allows soil resistivity to be measured simultaneously at three depths (here: 0–0.5 m, 0–1 m, and 0–1.7 m). Measurements were performed with a mobile device equipped with a differential Global Positioning System (GPS) to retrieve the geographical coordinates of samples with an accuracy of 20 cm. Measurements were made every 50 cm along lines spaced 2 m apart. This sampling scheme allowed for the description of soil resistivity with a very high spatial resolution (Fig. 1).

Earthworm sampling and DNA extraction

At each point of the grid, earthworms were sampled using a combination of formaldehyde extraction and hand-sorting. First, 10 l of 4‰ formaldehyde were applied onto a 1 m^2 surface, and earthworms expelled at the soil surface were collected during a 15-min period. Then, a soil volume of 25 cm × 25 cm × 25 cm and 30-cm depth was dug out in the centre of the square meter and hand sorted in the field. More details on the different species in the plots, sampling methodology, and species morphological identification are given in Richard *et al*. (2012). Specimens were fixed in pure alcohol until DNA extraction. A fragment of tegument was dissected and total genomic DNA was extracted using the DNeasy 96 Blood & Tissue Kit (Qiagen).

Molecular identification of *A. chlorotica* mitochondrial lineage

To determine the mitochondrial lineage to which each *A. chlorotica* individual belonged, we targeted the barcode portion of the cytochrome *c* oxidase I gene (*COI*). Some of the sequences have already been published in Dupont *et al*. (2011) (GenBank accession numbers: HM879975; HM417934–35, 37–41, 43, 45, 47–49, 52, 54, 55; HQ682441–46). For the other samples, a fragment of the *COI* gene was amplified according to Folmer *et al*. (1994). After purification using Microclean (Microzone Ltd), sequence reactions were performed using the BigDye Terminator Cycle Sequencing kit V. 1.1 (Applied Biosystems) and sequence data were obtained using a 3130xl Genetic Analyser (Applied Biosystems). Sequences were manually aligned using the BioEdit program (Hall,

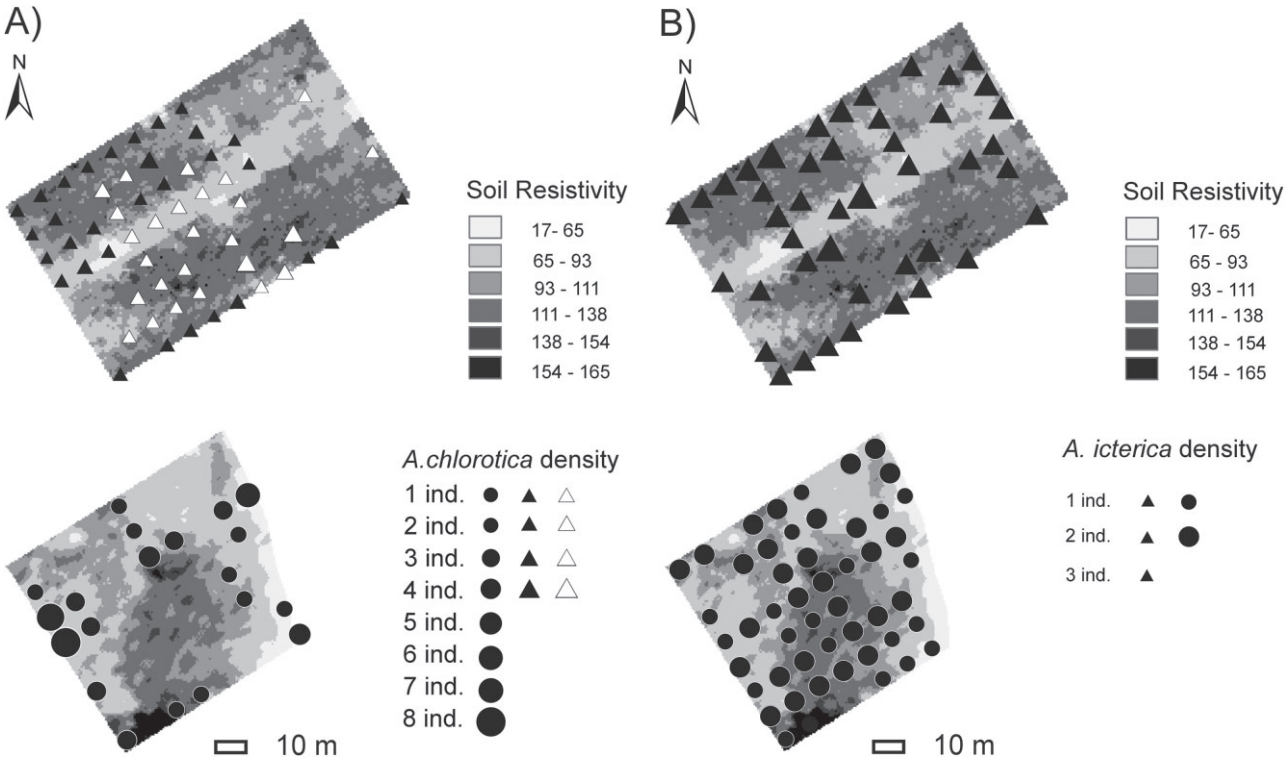

Figure 1. Distribution of the genotyped earthworms within P_A and P_B pastures, soil resistivity in Ohm-meter (Ωm), and Bayesian population structure clusters determined using GENELAND V 4.0.3 (Guillot *et al*., 2005), for *Allolobophora chlorotica* (A) and *Aporrectodea icterica* (B). Belonging to the various genetic clusters is represented by different geometrical forms.

1999). The GenBank accession numbers are KC569604–740.

In order to establish the correspondence of the generated sequences to the five lineages of the pink morph and the two lineages of the green morph of *A. chlorotica* uncovered in the studies by King *et al*. (2008) and Dupont *et al*. (2011), a phylogenetic analysis was performed using MEGA 6.0 software (Tamura *et al*., 2013). The sequences were aligned with all the haplotypes of King *et al*. (2008) and Dupont *et al*. (2011), and a phylogenetic tree was constructed by selecting the best-fit maximum-likelihood model in MEGA 6.0, based on the lowest Bayesian information criterion (BIC) score. The evolutionary distances were computed using the HKY + G + I as best-fit model with 1000 bootstrap replicate values. In addition, a local BLAST was implemented in the software BioEdit (Hall, 1999) using a nucleotide database formed by all the haplotypes of King *et al*. (2008) and Dupont *et al*. (2011). The match with the highest E-value was used to assign a lineage to the query sequence. Individuals that were assigned to the lineages of the pink morph were excluded from the analysis.

Microsatellite genotyping

Allolobophora chlorotica individuals were genotyped at eight microsatellite loci, as defined by Dupont *et al*. (2011). *Aporrectodea icterica* individuals were genotyped at the seven microsatellite loci described in Torres-Leguizamon *et al*. (2012) and one (Ai51) newly identified locus (M. Torres-Leguizamon and L. Dupont, unpubl. data; forward and reverse primer sequences: Ai51F 5' NED-AATCAACTGAACAGGCGTCC and Ai51R TTCGACAGAATGATTGTCCG). Loci [including Ai51 (annealing temperature 51 °C and 2.5 mM of $MgCl_2$)] were amplified by the polymerase chain reaction (PCR) following protocols detailed in Dupont *et al*. (2011) and Torres-Leguizamon *et al*. (2012). The migration of PCR products was carried out on a 3130xl Genetic Analyser using the LIZ500 size standard, and alleles were scored using GENESCAN V3.7 and GENOTYPER V3.7 software (Applied Biosystems).

Population genetic analyses

In each pasture plot, the genetic diversity of the two species was analysed by computing allele frequencies, number of alleles (N_{all}), and expected heterozygozity

(H_e) using GENETIX V 4.05 (Belkhir *et al*., 2004). To take into account variation in sample size, allelic richness (A_r; El Mousadik & Petit, 1996) was estimated using FSTAT v.2.9.3 (Goudet, 2000). The null hypothesis of independence between loci was tested from statistical genotypic disequilibrium analysis using GENEPOP V. 4.1.3 (Rousset, 2008). Evidence of null alleles was examined using the software MICRO-CHECKER (Van Oosterhout *et al*., 2004). Departure from Hardy–Weinberg expectations within plots were quantified by calculating the Weir & Cockerham's (1984) estimator of the fixation index, F_{is}, and conformity to Hardy–Weinberg equilibrium (HWE) was assessed with exact tests implemented in GENEPOP V. 4.1.3. To adjust for multiple comparisons, the false discovery rate (FDR) method (Benjamini & Hochberg, 1995), as implemented in the software SGoF (http://webs.uvigo.es/acraaj/SGoF.htm), was applied.

In order to investigate the genetic structure among populations, exact G tests of allelic differentiation were carried out between plots using GENEPOP V. 4.1.3. We also used a traditional population-differentiation approach based on F_{st} analysis. Weir & Cockerham's (1984) estimator of F_{st} was calculated using GENEPOP V. 4.1.3.

Spatial autocorrelation and gene dispersal

To investigate spatial genetic structure at the individual level, we conducted spatial autocorrelation analysis (Hardy & Vekemans, 1999), which provides a measure of genetic correlation as a function of Euclidean distances. Genetic distances between individuals were estimated using the Rousset's $\hat{a}$ estimator (Rousset, 2000) using SPAGEDI V1.3. To visualize the spatial genetic structure, we averaged pairwise genetic distance over a set of distance classes, and plotted it against the distance. Between 10 and 20 distance classes were used to equalize the number of comparisons among each distance class (approximately 200). Permutations (10 000) provided 95% confidence intervals about the null hypothesis of no spatial genetic structure.

If limited dispersal causes a decrease in genetic similarity between individuals at increasing geographical distances, Wright's IBD model and genetic neighbourhood (Wright, 1943; Wright, 1946) can be used to estimate the dispersal distance. Wright defines a neighbourhood size (N_b) as $4d_e\pi\sigma^2$, where d_e is the effective density and σ^2 is the mean squared axial parent–offspring dispersal distance (Wright, 1946). Assuming IBD within populations, σ^2 is estimated as $\sigma^2 = 1/(b_{\hat{a}}4d_e\pi)$, where $b_{\hat{a}}$ is the slope of the regression of genetic distance ($\hat{a}$) on the logarithm of geographical distance (Rousset, 2000). We estimated gene dispersal using the iterative procedure provided by SPAGeDi v. 1.3 (Hardy & Vekemans, 2002) but as it did not always converge, as it occurs in some cases according to the SPAGeDi manual, we simply used the relation $\sigma^2 = 1/(b_{\hat{a}}4d_e\pi)$.

Because the effective density of adult earthworms (d_e) is unknown, we used the total earthworm density d, d/2, d/5, and d/10 as alternative estimates of d_e. Thus, an upper and lower range of gene dispersal was obtained. We used earthworm densities obtained in Richard *et al*. (2012) for P_A (d = 22.80 and d = 19.94 ind m^{-2} for *A. chlorotica* and *A. icterica*, respectively) and unpublished data of Richard *et al*. for P_B (d = 8.18 and d = 36.27 ind m^{-2} for *A. chlorotica* and *A. icterica*, respectively).

Isolation by distance and isolation by resistance

In order to test the hypothesis of IBD and IBR, we used a traditional partial Mantel tests approach on the different explanatory and response distance matrices. The distance matrix of the response corresponded to the Rousset's $\hat{a}$ genetic distance between individuals. The distance matrix for IBD was computed as the geographical (Euclidean) distance between each pair of individuals. The distance matrix for IBR was computed as the cumulated cost of the least-cost path between each pair of individuals. Least-cost paths were produced with the Landscape Genetics Toolbox for Arcgis (Perry *et al*., 2010), which determines movement costs between pairs of points, based on a resistance matrix, represented here by the interpolated soil resistivity (kriged with a spherical model). We considered three scenarios to calculate costs of movements based on soil resistivity. In scenario 1, movement costs increased linearly with soil resistivity; in this case, movement costs at location xy corresponded to soil-resistivity values at xy: $cost_{xy} = R_{xy}$. In scenario 2, movement costs were lowest at intermediate values of soil resistivity; in this case, costs of movements were calculated as the absolute deviation from the average soil resistivity of the plot: $cost_{xy} = |R_{xy} - R^*|$, with R^* = average soil resistivity of the plot. In scenario 3, costs decreased with soil resistivity; in this case, $cost_{xy} = 1/R_{xy}$. Once movement costs were calculated at each location according to each scenario, we computed the least-cost path between each pair of points for each scenario. Then, we used partial Mantel tests to assess the likelihood of IBD and IBR following each scenario. These tests can evaluate the relationship between a response variable and an explanatory variable whilst controlling for the effect of a second explanatory variable, when these variables represent matrices of dissimilarity between pairs of locations (Legendre & Legendre, 1998). Tests were performed by permuta-

tion of the data. For instance, we estimated the effect of the geographical distance (D) while controlling the effect of movement costs (scenario 1) and denoted it D/R. We took advantage of this method in order to assess the likelihood of IBD and IBR in our data set, following each IBR scenario.

Bayesian clustering

Each study plot could enclose several subpopulations of each species. We investigated the occurrence of such cryptic population structure within plots using the Bayesian model implemented in GENELAND v.4.0.3 (Guillot, Mortier & Estoup, 2005) that simultaneously analyses spatial and genetic information. This model is based on HWE and linkage equilibrium (LE). We proceeded in two steps: a first run to infer the number of genetically distinct clusters (subpopulations) at each locality, K (i.e. true subpopulation number), and a second run with K fixed at the modal value from the first step to estimate the assignment of individuals to the inferred subpopulations. The first step was replicated five times to check for convergence, allowing K to vary from one to five clusters and using 10^6 Markov Chain Monte Carlo (MCMC) iterations. We used the correlated frequency model, which is predicted to be more powerful at detecting subtle differentiation. Moreover, the putative presence of null allele(s) was taken into account in the model and the spatial coordinates were treated as uncertain in order to allow samples with the same coordinates to be assigned to different subpopulations. In the second step undertaken when $K > 1$, we ran the MCMC five times again with K fixed, 10^6 MCMC iterations, and the other parameters unchanged. The runs were then post-processed in order to obtain posterior probabilities of subpopulation membership for each individual.

Because the correlated frequency model is prone to algorithm instabilities and particularly sensitive to departure from model assumptions (e.g. the presence of IBD), the Geneland manual recommends checking *ex-post* that the inferred groups are significantly differentiated. Thus, genetic differentiation between inferred clusters was checked using the exact G-test available in GENEPOP V4.1.3 (Rousset, 2008).

RESULTS

Genetic diversity

After excluding 11 individuals belonging to the pink morph and two individuals with ambiguous genotypes, the genotypes of 141 *A. chlorotica* individuals were analysed (95 and 46 individuals in P_A and P_B, respectively, Fig. 1). The same number of genotypes (141) was analysed in *A. icterica* (61 and 80 individuals in P_A and P_B, respectively, Fig. 1). All loci were polymorphic in both species, except for locus 2PE40, which was monomorphic in *A. icterica* in the P_B plot (AiP_B) (Table 1). Higher values of genetic diversity were obtained for *A. chlorotica* than for *A. icterica* (Table 1). Values of genetic-diversity indices within species were similar in both plots (Table 1). For example, identical values of $H_e = 0.530$ were obtained in both plots in *A. icterica*. In *A. chlorotica*, H_e was in the same range with estimates of 0.752 and 0.791 in P_A and P_B, respectively.

Hardy–Weinberg equilibrium and linkage equilibrium

In *A. icterica*, all loci were unlinked. In contrast, in *A. chlorotica* one pair of loci (Ac476–Ac419) was out of LE in the P_A plot (AcP_A) and seven pairs departed significantly from LE in the P_B plot (Ac127–Ac418, Ac127–Ac476, Ac127–Ac529, Ac418–Ac170, Ac418–Ac419, Ac418–Ac476, and Ac418–Ac528). In their study of the genetic structure of the *A. chlorotica* aggregate in Europe, Dupont *et al*. (2011) showed no linkage among these loci, except for Ac127–Ac476 in one of the populations. Thus, these departures from LE are probably explained by genetic sub-structure within the plots (see Ohta, 1982) rather than by physical linkage between loci. Nevertheless, the locus Ac476 (less informative than Ac419) was excluded from the AcP_A data set, and Ac418 and Ac127 were excluded from the AcP_B data set for the clustering analyses that required LE.

Heterozygote deficiency, indicated by significant deviation from HWE, was observed for several *A. chlorotica* loci (Table 1). The existence of null alleles was suggested by Microchecker results for some of the *A. chlorotica* loci (Table 1), but they were different in the two plots. Estimated frequencies of null alleles for these loci were relatively low, ranging from 6.1% to 11.2%. In *A. icterica*, deviations from HWE were revealed for loci 2PE70 and C4 in both populations (Table 1). Although no null allele was detected in P_B for the 2PE70 locus, the estimated frequency of null alleles was particularly high for the locus C4 in both plots (32.7–36.9%). Thus, this locus was excluded from all other analyses.

Gene flow within plots

Significant genetic structure was revealed at the population level, with F_{st} values (0.018 and 0.014 in *A. icterica* and *A. chlorotica*, respectively) associated with significant exact tests ($P < 0.001$). The spatial genetic structure at the individual level was investigated through IBD and autocorrelation analyses.

Table 1. Genetic characteristics of each plot for both species

Species	Plot	N_{ind}	N_{all}	A_r	H_e	F_{is} (null)								
						Ac127 [15]	Ac170 [21]	Ac418 [14]	Ac419 [6]	Ac476 [6]	Ac527 [7]	Ac528 [11]	Ac529 [13]	All
A. chlorotica	P_A	95	10.5	9.3	0.752	**0.135** (0.061)	0.063	**0.126** (0.063)	0.110	0.032	**0.025**	**0.079**	**0.236** (0.112)	**0.104**
	P_B	46	9.5	9.5	0.791	0.028	**0.109**	0.006	0.238 (0.107)	0.057	**0.041** (0.097)	**0.210**	0.103	**0.098**
A. icterica	P_A	61	3.6	3.6	0.530	0.130	0.204	−0.041	0.131	0.254 (0.118)	−0.004	**0.389** (0.169)	**0.815** (0.369)	**0.268**
	P_B	80	3.5	3.4	0.530	0.077	0.038	−0.056	0.033	−0.023	–	**0.047**	**0.706** (0.327)	**0.134**
						Ai45 [4]	Ai51 [4]	Ai56 [3]	Ai68 [6]	2PB10 [3]	2PE40 [3]	2PE70 [3]	C4 [5]	All

The following are given for each sample: A_r, allelic richness; H_e, gene diversity; N_{all}, number of alleles; and N_{ind}, sample size (individuals) for genetic analysis. The estimator of the fixation index (F_{is}) is also given for each sample, with the total number of alleles for each locus within square brackets, significant values for heterozygote deficiency (after FDR correction) in bold, and null-allele frequency estimated by MICROCHECKER software in parentheses.

A weak, but significant, relationship between genetic distances and the logarithm of geographical distances was obtained for *A. chlorotica* in P_B (slope of the regression = 0.0068, $P < 0.001$). This IBD (Table 2) was confirmed by the autocorrelation analysis (Fig. 2). Considering d as the upper limit of effective density and d/10 as the lower limit of effective density, the lowest estimate of gene dispersal (i.e. parent–offspring dispersal) distance was therefore 3.41 m and 3.78 m in AcP_B. Considering two generations of *A. chlorotica* during 1 year in Normandy, an approximate dispersal rate ranging from 6.81 to 7.56 m for 1 year may be estimated for this species. An IBD was also suggested in AcP_A when the geographical distances were partialed out by soil resistivity (D/R, $P < 0.001$, Table 2). Whereas no IBD was detected in *A. icterica* populations using all genotypes (Table 2), correlograms suggested a positive relationship between genetic and geographical distance in the restricted range of 20–61 m (Fig. 2). However, the gene-dispersal distance could not be estimated for this species because of the low level of polymorphism of the microsatellite loci (H_e ranged from 0.000 to 0.716). Indeed, Leblois, Estoup & Rousset (2003) recommended using loci with H_e of around 0.7 to maximize the efficiency of the estimation of σ^2.

Results of partial Mantel tests suggest that soil properties might play a role in the structuring of genetic variation at the scale of the plot. Indeed, IBR was shown in AcP_A, AcP_B, and AiP_B (Table 2).

Genetic sub-structure within plots

The occurrence of cryptic population structure was suggested in both plots for *A. chlorotica* by the Geneland Bayesian analysis that identified two groups of individuals in AcP_A (Fig. 2) and five groups in AcP_B. The clusters AcP_A–C_1 (cluster 1 of the *A. chlorotica* P_A plot) and AcP_A–C_2 (cluster 2 of the *A. chlorotica* P_A plot) were composed of 42 and 53 individuals, respectively, and were significantly differentiated (exact G-test, $P < 0.001$). The genetic differentiation between AcP_A–C_1 and AcP_A–C_2 clusters ($F_{st} = 0.016$) was higher than between AcP_A–C_1 and the P_B plot ($F_{st} = 0.014$, $P < 0.001$) but lower than between AcP_A–C_2 and P_B ($F_{st} = 0.022$, $P < 0.001$).

The five clusters identified in P_B were composed of 2, 12, 13, 9, and 10 individuals. These small population sizes prevented robust testing of genetic differentiation. Moreover, the Bayesian spatial correlated model used in Geneland is particularly sensitive to the presence of IBD (see the Geneland manual, Guillot *et al.*, 2005; Frantz *et al.*, 2009). Hence, the five detected clusters are probably misleading results.

Table 2. Results of the partial Mantel tests to assess isolation-by-distance (IBD) and isolation-by-resistance (IBR) hypotheses with the geographical distance (D), the distance based on raw soil resistivity (R), and the average soil resistivity (R*)

Species	Plot	Predictor	Mantel r	*P*-value		IBD or IBR
A. chlorotica	P_A	D	−0.04	0.002	*	
		R	−0.07	$< 10^{-4}$	*	
		D/R	0.1	$< 10^{-4}$	*	IBD
		R/D	−0.12	$< 10^{-4}$	*	
		1/R	−0.03	0.04	*	
		D/(1/R)	−0.1	$< 10^{-4}$	*	
		(1/R)/D	0.1	$< 10^{-4}$	*	IBR
		|R-R*|	−0.03	0.01	*	
		D/|R-R*|	−0.03	0.03	*	
		|R-R*|/D	0.003	0.4	ns	
A. chlorotica	P_B	D	0.12	$< 10^{-4}$	*	IBD
		R	0.13	1.10^{-4}	*	IBR
		D/R	−0.03	0.14	ns	
		R/D	0.06	0.028	*	IBR
		1/R	0.11	$< 10^{-4}$	*	IBR
		D/(1/R)	0.04	0.08	ns	
		(1/R)/D	−0.02	0.3	ns	
		|R-R*|	0.08	0.004	*	IBR
		D/|R-R*|	0.09	0.002	*	IBD
		|R-R*|/D	−0.015	0.3	ns	
A. icterica	P_A	D	0.02	0.17	ns	
		R	0.04	0.04	*	IBR
		D/R	0.014	0.27	ns	
		R/D	0.09	0.34	ns	
		1/R	0.05	0.02	*	IBR
		D/(1/R)	0.03	0.06	ns	
		(1/R)/D	−0.03	0.08	ns	
		|R-R*|	0.05	0.02	*	IBR
		D/|R-R*|	−0.01	0.3	ns	
		|R-R*|/D	0.03	0.08	ns	
A. icterica	P_B	D	−0.05	0.001	*	
		R	−0.06	$< 10^{-4}$	*	
		D/R	−0.019	0.13	ns	
		R/D	0.003	0.44	ns	
		1/R	−0.05	0.001	*	
		D/(1/R)	−0.02	0.19	ns	
		(1/R)/D	0.002	0.43	ns	
		|R-R*|	−0.06	$< 10^{-4}$	*	
		D/|R-R*|	−0.02	0.16	*	
		|R-R*|/D	−0.04	0.012	*	

The formula X_1/X_2 is used when the effect of X_1 is tested while controlling the effect of X_2.
*Significant value; ns, non-significant value.

DISCUSSION

The polymorphism of microsatellite DNA sequences varied across loci and across species. In particular, microsatellite markers used for genotyping *A. chlorotica* individuals were more polymorphic than the markers used for *A. icterica*. For instance, the number of alleles was three times higher for *A. chlorotica* than for *A. icterica*. This difference is not a particularity of the study because the level of genetic diversity recorded is similar to the results of Dupont *et al*. (2011) for *A. chlorotica* and of Torres-Leguizamon *et al*. (2012) and Torres-Leguizamon *et al*. (2014) for *A. icterica* at the landscape/region scale. In our study, *A. icterica*

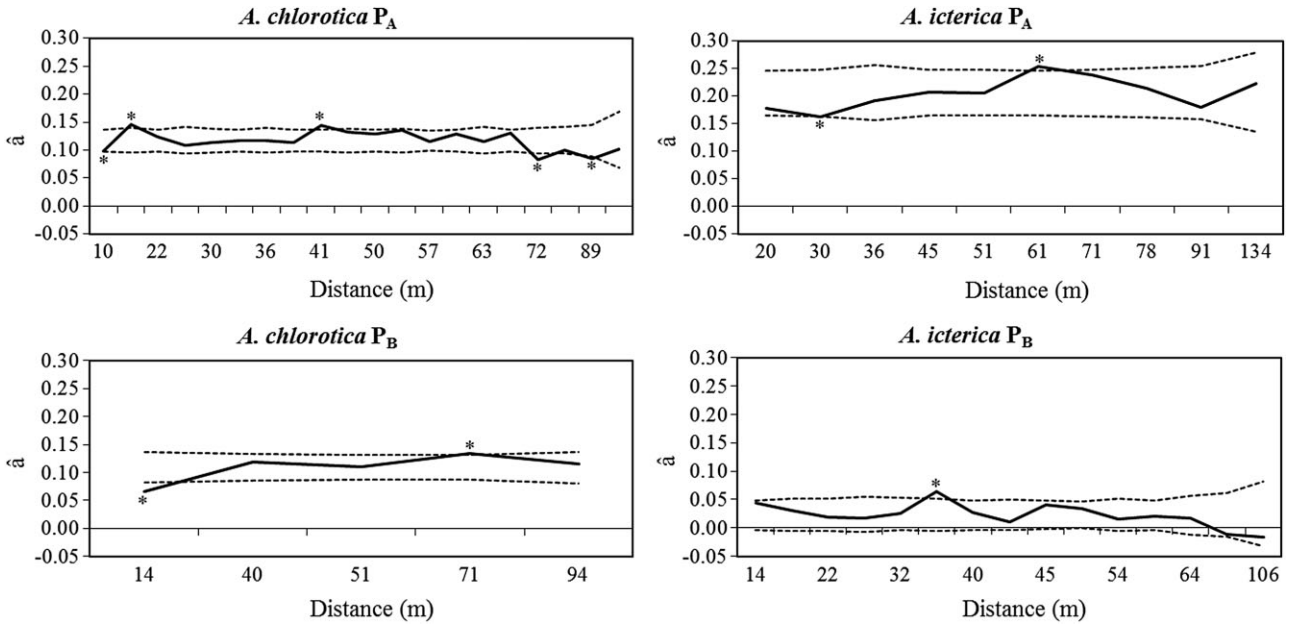

Figure 2. Correlograms (solid lines) of estimated $\hat{a}$ genetic distance (Rousset, 2000) for both plots and both species. Dotted lines indicate the 95% null hypothesis confidence region. Significant values: $^*P < 0.05$.

microsatellites displayed lower polymorphism than all other microsatellites obtained for other earthworm species using similar isolation strategies. For instance, the N_{all} value of earthworm's microsatellite loci typically ranges from five to 17 (review in Torres-Leguizamon *et al*., 2014), whereas *A. icterica* loci display a maximal N_{all} of 3.6. Such a low polymorphism of molecular markers decreases the precision in estimates of heterozygote deficiencies (Robertson & Hill, 1984), statistical tests of differentiation (Goudet *et al*., 1996), and estimates of gene-dispersal distances (Leblois *et al*., 2003). The low polymorphism of *A. icterica* microsatellite loci could be reflecting bottlenecks in the evolutionary history of this species (Torres-Leguizamon *et al*., 2014). More research is needed in order to understand, more clearly, this particularity of *A. icterica* simple sequence repeats.

Most of the loci were at LE, except for one pair of loci in the P_A plot and several pair of loci in the P_B plot for *A. chlorotica*. In a single random-mating population, linkage disequilibrium or nonrandom association of alleles between two loci may be produced by epistatic interaction in fitness between the loci concerned (Kimura, 1956) and random genetic drift as a result of to finite population size (Ohta & Kimura, 1969). Because the markers used are supposedly neutral, the hypothesis of epistatic interaction may be ruled out. Random fluctuation of gamete frequencies as a result of genetic drift is enhanced if the population is subdivided or if mating were not random in the population (review in Ohta, 1982). Thus, linkage disequilibrium within *A. chlorotica* samples could be explained either by sub-structure within the population (i.e. the Wahlund effect) or by mating among relatives (i.e. inbreeding).

Such phenomena (the Wahlund effect and/or inbreeding) should have resulted in deviation from HWE. Significant deficits of heterozygotes were indeed observed in both plots for both species. In *A. icterica*, deviations from HWE were largely caused by null alleles at the C4 locus. Null allele existence was also suggested at several *A. chlorotica* loci, but at a low frequency. Our results showed that sub-structure within plots and/or mating among relatives also contribute to the deviation from HWE in this species.

Sub-structure was suggested by the Bayesian analysis of genetic clustering which revealed two clusters within the P_A plot for *A. chlorotica*. It is noteworthy that the upper half of P_A was ploughed 1 year before sampling, whereas the lower half was not. It is thus proposed that habitat perturbation because of human activities might be responsible for the *A. chlorotica* spatial genetic clustering at this fine geographical scale. Ploughing might thus alter the genetic structure of earthworm populations for at least 1 year. This perturbation could probably be even longer for *A. chlorotica* because of the limited dispersal capacity of the species. This result suggests that natural and artificial (i.e. caused by human activity) habitat spatial heterogeneity can be an important contributor to earthworm population genetic structure.

Mating among relatives seems likely for *A. chlorotica* in at least one of the plot. In P_B, the spatial autocorrelation analysis revealed a pattern of fine-scale genetic structure with restricted gene dispersal for this species. The estimation of gene-dispersal distance during one generation (approximately 6 months) ranged from 3.41 to 3.78 m. This estimation represents a dispersal ranging from 6.82 to 7.56 m for 1 year. This is close to the estimation of 4 m $year^{-1}$ previously obtained for the annual dispersal rate of *A. chlorotica* during colonization of worm-free clayey polder soils in the Netherlands (review in Eijsackers, 2011). Such a restricted dispersal confirms that there is a higher probability that individuals mate with individuals born in close proximity to themselves than with individuals born far away, a pattern favouring inbreeding. A similar pattern was revealed by Novo *et al*. (2010a), who investigated the mating strategy of *Hormogaster elisae*, an outcrossing endogeic earthworm endemic to the Central Iberian Peninsula. Their results suggested that individuals of *H. elisae* were rather sedentary and did not relocate over long distances to find mating partners.

In contrast to *A. chlorotica*, neither IBD nor genetic clustering was detected within plots for *A. icterica.* This low signal of genetic structure may be explained by an important dispersal capacity of the species and/or by the low polymorphism of the microsatellite loci that could have prevented the detection of subtle genetic differentiation. A similar absence of relationship between geographical and genetic distance was shown at a regional scale in this species (Torres-Leguizamon *et al*., 2014). In their review on earthworm genetic structure, Costa *et al*. (2013) asserted that most of the few studies on genetic structure of earthworms found no relationship between genetic and geographical distances. In contrast, Novo *et al*. found a pattern of isolation according to distance at both interpopulation (Novo *et al*., 2009) and intrapopulation (Novo *et al*., 2010a) levels in hormogastrid earthworms. Altogether, these studies were not achieved at the same geographical scale, did not use similar molecular markers [e.g. amplified fragment length polymorphisms (AFLPs), microsatellites, or the *COI* gene], and targeted species belonging to various eco-morphological groups (i.e. anecic and endogeic) and having various reproductive strategies (i.e. amphimixis and parthenogenesis); thus, it is difficult to draw general conclusions about earthworm spatial genetic variation.

Experimental work has shown that environmental properties are strong determinants of dispersal in earthworms and thus predicted that their spatial distribution should be correlated with environmental data (Mathieu *et al*., 2010). To date, only a few studies have investigated the relationship between environmental data, such as soil characteristics, and the spatial genetic structure of earthworm populations (Lentzsch & Golldack, 2006; Novo *et al*., 2010b). Here, despite numerous significant partial Mantel tests at the scale of the whole study, little consensus has emerged regarding the direction of the relationship between genetic distances, geographical distances, and soil resistivity because geographical distances and soil resistivity, when not partialed out by the other matrix, were not always positively related to genetic distances. The significant patterns of IBR were thus difficult to interpret, in particular for *A. icterica* in the plot P_A. In previous studies at a larger spatial scale, Lentzsch & Golldack (2006) found no relationship between the distribution of *A. caliginosa* genotypes and soil properties (e.g. pH, soil organic carbon, total nitrogen, and clay content) along a 151-m transect, and Novo *et al*. (2010b) found a weak relationship between soil texture (i.e. coarse sand and total loam content) and genetic distances between populations of hormogastrid earthworms at a similar scale ($< 100\ km^2$). Altogether, these results reveal that there is no simple relationship between soil properties and earthworm genetic structure, and suggest that other factors, such as demographic events (i.e. population bottleneck events and genetic drift), may be particularly important in shaping the genetic composition of earthworm populations.

CONCLUSION

Only scarce data are available on the fine-scale population structure of soil invertebrates. To our knowledge, only two studies describe genetic patterns of soil macro and meso invertebrates using individual-based approaches: the study of Sullivan, Dreyer & Peterson (2009), showing that the collembolan *Folsomia candida* exhibits genetic population structuring over a very fine geographical scale ($0.65\ km^2$), and our study.

Here, we showed that in a 42-year-old pasture without recent perturbation, *A. chlorotica* displayed a neighbourhood structure of randomly mating earthworms, in which neighbour individuals tend to be more genetically similar to one another, whereas no limit to gene flow was detected for *A. icterica*. In the other plot, where tillage had recently deteriorated the physical conditions of the soil, the expected pattern of IBD in *A. chlorotica* seemed to be erased, whilst a pattern of IBR was revealed for *A. icterica*. Thus, the present study emphasizes that agricultural practices contributing to a fragmentation of the species habitat may durably alter the population genetic structure of earthworms at a very small scale.

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Annexe 6

Mathieu, J., M. Grimaldi, P. Jouquet, C. Rouland, P. Lavelle, T. Desjardins, and J.-P. Rossi, Spatial patterns of grasses influence soil macrofauna biodiversity in Amazonian pastures. Soil Biology and Biochemistry, 2009. 71: p. 586-593.

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Spatial patterns of grasses influence soil macrofauna biodiversity in Amazonian pastures

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ABSTRACT

Grasslands are often characterized by small-scale spatial heterogeneity due to the juxtaposition of grass tufts and bare ground. Although the mechanisms generating plant spatial patterns have been widely studied, few studies concentrated on the consequences of these patterns on belowground macrofauna. Our objective was to analyze the impact of grass tuft (*Brachiaria bryzantha* cv. *marandu*) spatial distribution on soil macrofauna diversity in Amazonian pastures, at a small scale (less than 9 m^2). Soil macrofauna was sampled among *B. bryzantha* tufts, which showed a variable spatial distribution ranging from dense to loose vegetation cover. The vegetation configuration explained 69% of the variation in total soil macrofauna density and 68% of the variation in total species richness. Soil macrofauna was mainly found in the upper 10 cm of soil and biodiversity decreased with increasing distances to the nearest grass tuft and increased with increasing vegetation cover. The size of the largest grass tuft and the microlandscape connectivity also had a significant effect on biodiversity. The density and species richness of the three principal soil ecological engineers (earthworms, ants and termites) showed the best correlations with vegetation configuration. In addition, soil temperature significantly decreased near the plants, while soil water content was not influenced by the grass tufts. We conclude that soil macrofauna diversity is low in pastures except close to the grass tufts, which can thus be considered as biodiversity hotspots. The spatial arrangement of *B. bryzantha* tussocks influences soil macrofauna biodiversity by modifying soil properties in their vicinity. The possible mechanisms by which these plants could affect soil macrofauna are discussed.

1. Introduction

Large-scale determinants of soil macrofauna diversity are relatively well known: climate, soil type, land-use management practices and landscape structure are among the most influential factors (Dauber et al., 2003, 2005). At smaller scales, however, there is much less agreement about the environmental factors that drive soil macrofauna diversity and distribution (Lavelle and Spain, 2001). It has been suggested that in general, grassland invertebrates are less likely to be limited by the quantity of food available, but rather by microclimate and food quality (Curry, 1994). Microclimate is very important since the body temperature of soil macrofauna varies with external conditions (thermoconformers) and the range tolerated by many species is quite narrow (Precht et al., 1973; Geiger and Aron, 2003). In addition, soil macrofauna must maintain body water content within fairly narrow limits, which creates a dependence on water. Soil macrofauna organisms are also sensitive to the nutrient content of their food because they need to maintain their internal chemical concentrations and the balance between the different nutrients of their body within a strict range (Sterner and Elser, 2002; Martinson et al., 2008). Thus elements of food quality, such as phosphorus (Kay et al., 2006; McGlynn and Salinas, 2007), nitrogen (Warren and Zou, 2002) or Ca^{2+} (Reich et al., 2005) content, can become a limiting factor. As autogenic ecosystem engineers, plants modify food quality, quantity, and the microclimate of soil macrofauna. With their associated microflora they affect the physical and chemical properties of their environment by producing and taking up organic and mineral substances, creating biopores, and producing litter (Lavelle and Spain, 2001). Plants modify the microclimate in their vicinity by cooling down the soil and air in the shade of their leaves. They also modify

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humidity by intercepting wind and rain, and by absorbing water in the ground. As a consequence, they create specific living conditions (i.e. physical habitats and available food for e.g., Jackson and Caldwell, 1993). A wealth of literature deals with the consequence of these engineering effects on microbial communities (Spetch, 1958; Northup et al., 1999) but much less is known about the relationships between vegetation cover and soil macrofauna diversity and distribution.

In Amazonian pastures, vegetation is typically dominated by large herb tufts of the genus *Brachiaria*, which clearly alternate with bare ground. The vegetation cover is highly variable, from dense to loose, which leads to heterogeneous habitats for soil organisms. Cattle ranching is the dominant activity in Amazonia in terms of land surface (Muchagata and Brown, 2003) and the major motivation for deforestation. Pastures are often characterized by a dramatic decrease in productivity after 10 years of exploitation (Costa and Rehman, 1999; Muchagata and Brown, 2003). This phenomenon is accompanied by a reduction in soil macrofauna biodiversity (Fragoso et al., 1997; Barros et al., 2002). Soil macrofauna biodiversity plays a recognized role in the productivity and soil functioning of these systems (Chauvel et al., 1999; Laossi et al., 2008), but the factors that drive its distribution are still poorly documented. In particular we lack information about the small-scale sources of environmental variability that cause local patterns of soil macrofauna biodiversity (Mathieu et al., 2004).

Our aim was to analyze the effect of vegetation spatial configuration on belowground soil macrofauna density and species richness in Amazonian pastures. We investigated the correlations between the spatial configuration of *Brachiaria bryzantha*, a very common plant in these pastures, and soil macrofauna distribution, and the relations between the spatial configuration of *B. bryzantha* and the soil macrofauna environment. In particular, we discuss the role of soil temperature and water content as factors, which structure the microenvironment, and their possible consequences on soil macrofauna diversity and abundance.

2. Materials and methods

2.1. Site

This study was carried out in a community of smallholders in southeast Amazonia, at the Benfica Field Station (5°16′ S and 49°50′ E, Pará, Brazil). We surveyed three, 6 years old pastures of 20 ha on average, planted with the perennial African grass *B. bryzantha* cv. *Marandu*, the most common species used in this area. Pastures mainly served for cattle ranching. *B. bryzantha* forms massive tufts reaching 0.8 m in diameter that can locally have a fairly even spatial distribution and are separated by bare ground, leading to a heterogeneous vegetation cover (Fig. 1 shows an average configuration). In the pastures under study, grasses were planted individually when the pasture was established. The climate is tropical humid with an annual rainfall of 1800 mm and an average temperature of 26 °C. The rainy season generally starts in November or December and ends during May or June. Clayey Ferralsol soils (Isss, 1998) are dominant with varying thicknesses of aggregated, macroporous and permeable horizons, above compact alterites (subsoil). They are acid (pH = 5.8) and contain 12.7 g kg^{-1} of C, 1.8 cmolc kg^{-1} of Ca^{2+}, 5.0 mg kg^{-1} of P on average in the 10 upper cm.

2.2. Sampling design and procedures

2.2.1. Soil macrofauna

The soil macrofauna was sampled by taking 60 evenly distributed samples along 6 transects in 3 pastures (2 transects per pasture, 10 m between each sample). The sampling design was part of a wider campaign to sample soil macrofauna at the landscape level (Mathieu et al., 2005). Soil macro-organisms were collected following the tropical soil biology and fertility method (Anderson and Ingram, 1993). At each sampling point, an area of 25 × 25 × 30 cm deep was excavated and the surface cover directly above the sample was either classified as "bare ground" or "microsite"(when there was a grass tuft or dead tree trunk on the ground). The corresponding variable is hereafter referred to as "Sample Type" (ST). The litter layer and soil were quickly removed before the macroinvertebrates were hand-sorted and preserved in 4% formalin solution. In the laboratory, adult invertebrates were classified into 7 broad taxonomic groups: earthworms, termites, ants, spiders, coleoptera, centipedes and millipedes and identified at the species level with the help of a number of taxonomists. Individuals of other groups were pooled as a single group called "others". Samples were taken at the end of the rainy season in 2002 when communities were presumed to be at peak abundance and biomass (Anderson and Ingram, 1993). Macrofauna extracted from soil and litter layers was combined in the analyses.

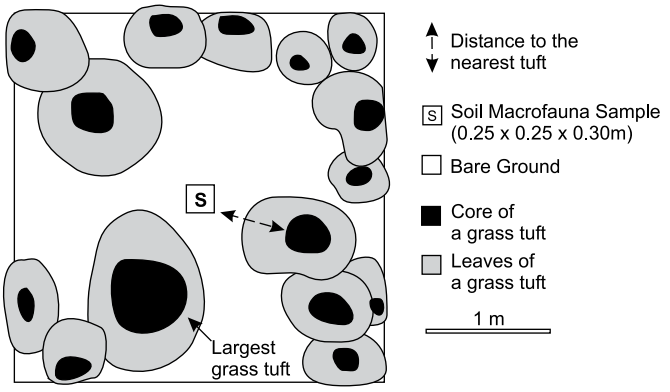

Fig. 1. A typical 9 m^2 map of the vegetation cover illustrating how the configuration of the grass tufts results in a micro-landscape. Grass tufts can be separated into two sections: the core of the tufts (i.e. the basal area), and the area occupied by the leaves (i.e. the canopies. Only the basal areas were used for calculating micro-landscape metrics.

2.2.2. Quantifying the vegetation spatial organization

The vegetation cover around each sample was described within a squared area of 9 m^2 centered on the sample (Fig. 1). Strings were attached to the ground to form a regular grid of 0.3 m × 0.3 m and the soil cover was mapped at a scale of 1:20 to show grass tussocks, grass canopies and the presence of microhabitats such as dead wood, cattle dung and termite mounds. The maps were then digitalized and rasterized (resolution: 0.1 m × 0.1 m per pixel). This produced simple micro-landscape maps with 2 strata: bare soil (matrix) and grass tufts (patches). The resulting "micro-landscapes" were described by four classical landscape metrics (Giles and Trani, 1999): the percentage of soil occupied by vegetation (PL), the area of the largest grass tuft in the area (LPI, m^2), the Edge Density (ED, m m^{-2} i.e. the length of the vegetation boundary, in meter, per square meter of area) and the Patch Density (PD, ind m^{-2}, i.e. the number of grass tufts per unit area). Only the central part of the tufts (corresponding to the stems, or "basal area", Fig. 1) was considered because these vary considerably less with time compared to the whole leaf system which is grazed by cattle. The distance between the soil macrofauna sample and the nearest grass tuft was also measured. The metrics were calculated using Fragstats (McGarigal and Marks, 1995). In addition we evaluated visually the amount of dead wood on the ground within the area of 9 m^2, and classified it as 0: no wood, 1: some twigs and branches, 2: big branches or trunk. We will refer to this variable as WOOD here in.

2.2.3. Microclimate

Horizontal soil temperature and water content patterns were studied in two quadrats in one of the pastures, with one quadrat of 1 m^2 and another of 9 m^2. Different quadrat sizes were used because it was not possible to determine in advance which size was best suited to assess the soil temperature and water content variability. Measurements were taken in regular grids of 0.1 and 0.3 m mesh, for plots of 1 m^2 and 9 m^2, respectively, giving 100 measurements in each unit. The average temperature of the upper top 15 cm of soil at each point was recorded using a high precision temperature probe. The water content expressed as the volume of water per volume of soil was measured at exactly the same points using time domain reflectometry (TDR: Dalton et al., 1984; Teixeira et al., 2003). In a separate experiment, a vertical profile of soil temperature was also recorded below and around one isolated grass tuft. Measurements were made at regular intervals at 2, 5, 10 and 20 cm depth and every 5 cm horizontally, over 1 m. Measurements started from below the centre of an isolated grass tuft and spread toward bare ground. The radius of the grass tuft's tussock was 15 cm while the canopy reached 35 cm in radius. Measurements were made at midday, when the air temperature was high (37 °C), in May.

2.3. Statistical analyses

2.3.1. Relationship between vegetation cover configuration and soil macrofauna

The relationships between the vegetation spatial organization and macrofauna were explored using backward stepwise multiple regressions. Soil macrofauna density and species richness were $\log(x+1)$ transformed and were entered as the dependent variables, while vegetation metrics ("PL", "ED", "PD", "LPI"), sample type ("ST" in the tables), presence of wood on the ground ("WOOD" in the tables), and distance to the nearest grass tuft ("DIST") were entered as explanatory variables. All variables and their interaction with the sample type (ST) were included in the analysis. All non-significant effects were removed step by step to produce models containing only significant effects (with alpha = 0.05) and minimum AIC (Burnham and Anderson, 2002). Finally, the models were compared with the results of automatic stepwise multiple regression to check for robustness. The Table 2 shows r^2 adjusted by the number of variables. Residuals were analyzed carefully to check for homogeneity of variance, normality and the influence of individual observations. Computations were made using R software (R Development Core Team, 2007).

2.3.2. Spatial pattern of soil temperature and water content

The spatial pattern of soil temperature and water content was assessed by variogram analysis (Rossi et al., 1995; Goovaerts, 1997) and interpolation by point kriging (Isaaks and Srivastava, 1989). Semi-variograms were computed using GSTAT (Pebesma and Wesseling, 1998), with the smallest lag distance equal to the mesh size and the largest lag set to half the maximum distance between sampling points (Isaaks and Srivastava, 1989). The areas where temperature and water content were measured were mapped to calculate the distance to the nearest grass tuft and examine its influence on the measurements using simple regressions.

3. Results

3.1. Differences of soil macrofauna between bare ground and microsites

Sample location had a major effect on the macrofauna species richness and density (Table 1). The overall species richness was double that in microsites (nine to ten species per sample) than under bare ground (four species per sample). The overall density was treble that in microsites (762 ind m^{-2}) than under bare ground (195 ind m^{-2}). All groups presented the same trend, either in terms of species richness or density. The species composition was also very different between bare ground and microsites: the proportion of shared species was 16% and 17% between bare soil and herb tufts or dead trunks, respectively, whereas it was 28% between herb tufts and dead trunks. Termites were dominated by *Amitermes*, *Heterotermes* and *Cornitermes*, ants were dominated by the genus *Hypoponera*, and earthworms were dominated by a species of *Andiorrhinus*.

3.2. Relationship between soil macrofauna and the spatial organization of the vegetation cover

Stepwise multiple regression analyses for species richness and density are summarized in Table 2. The vegetation configuration explained 69% of the variation in total soil macrofauna density and 68% of the variation in total species richness. In the model, total species richness increased when the vegetation cover (AREA) increased and decreased with increasing distance to the nearest grass tuft (DIST). In bare ground, species richness also decreased with increasing edge density (ED). Total density decreased with increasing distance to the nearest grass tuft (DIST) and increased with the size of the largest grass tuft (LPI). In microsites, density increased with increasing edge density (ED), while in bare ground it decreased with increasing ED.

Considered separately, the diversity and density of all groups of soil macrofauna varied significantly according to the spatial configuration of the vegetation (Table 2). The strongest relationships were obtained for termite density ($r^2 = 0.64$) and earthworm species richness ($r^2 = 0.38$). The weakest relationships were obtained for spiders ($r^2 = 0.07$ for species richness and density). The distance to the nearest grass tuft (DIST) was the most influential micro-landscape variable, affecting all groups except earthworms and centipedes, and was always negatively correlated to the density or the species richness. Edge density (ED) was the second most influential variable. It was generally negatively correlated to density or species richness in bare ground, whereas it was positively correlated in microsites. It had significant influence on ants, termites, and centipedes. The third most important variable was the amount of wood (WOOD), which had always a positive effect on biodiversity. It increased termites and millipedes species richness and density. The size of the largest grass tuft (LPI) was always correlated positively to biodiversity, at the exception of ant density in microsites. It influenced significantly earthworms' species richness, ants' density, and termites' species richness. The vegetation cover (AREA) was positively correlated with ants'

Table 1
Species richness (number of species) and density (ind m^{-2}) per sample (standard error in brackets) of the different groups, below microsites and under bare ground.

	Microsites				Bare ground			
	Species richness		Density		Species richness		Density	
earthworms	1.7	(0.2)	109.3	(21.0)	0.8	(0.1)	30.9	(8.9)
ants	2.0	(0.2)	159.3	(38.0)	1.3	(0.3)	73.1	(24.2)
termites	0.7	(0.1)	326.7	(137.8)	0.2	(0.1)	20.3	(14.5)
coleoptera	1.4	(0.2)	36.7	(6.9)	0.9	(0.2)	24.5	(9.2)
spiders	0.4	(0.1)	7.3	(2.5)	0.1	(0.1)	2.1	(1.0)
centipedes	0.4	(0.1)	14.7	(5.3)	0.1	(0.1)	1.6	(0.9)
millipedes	0.5	(0.2)	16.7	(6.9)	0.1	(0.1)	2.1	(1.0)
Others	4.6	(0.8)	129.3	(29.0)	1.1	(0.3)	44.2	(23.0)
All together	9.5	(0.9)	764.0	(146.3)	4.0	(0.7)	194.7	(54.3)

Table 2
Standardized coefficients of the linear models for species richness and density on environmental variables. Global fit of the model is indicated by the adjusted coefficient of determination (r^2aj). For abbreviations see Material and methods.

Group	Dependant variable	Sample type	Coefficients of the linear model
earthworms			
	Species Richness (ln)	Bare Ground	$0.51 + 0.16 \times LPI - 0.14 \times PD$
	$r^2aj = 0.33$	Microsite	$1.02 + 0.01 \times LPI - 0.14 \times PD$
	Density (ln)	Bare Ground	$0.78 - 0.22 \times PD$
	$r^2aj = 0.38$	Microsite	$1.73 - 0.22 \times PD$
ants			
	Species Richness (ln)	Bare Ground	$0.8 - 0.25 \times DIST - 0.19 \times ED$
	$r^2aj = 0.41$	Microsite	$0.8 - 0.25 \times DIST - 0.19 \times ED$
	Density (ln)	Bare Ground	$1.22 - 0.45 \times DIST + 0.30 \times AREA + 0.17 \times LPI$
	$r^2aj = 0.25$	Microsite	$1.63 - 0.45 \times DIST + 2.0 \times AREA - 1.51 \times LPI$
termites			
	Species Richness (ln)	Bare Ground	$0.29 - 0.15 \times DIST + 0.10 \times WOOD$
	$r^2aj = 0.64$	Microsite	$0.29 - 0.15 \times DIST + 0.10 \times WOOD$
	Density (ln)	Bare Ground	$0.21 + 0.19 \times LPI + 0.05 \times ED + 0.24 \times WOOD$
	$r^2aj = 0.27$	Microsite	$1.73 + 1.04 \times LPI + 1.27 \times ED + 0.24 \times WOOD$
coleoptera			
	Species Richness (ln)	Bare Ground	$0.62 - 0.23 \times DIST$
	$r^2aj = 0.19$	Microsite	$0.62 - 0.23 \times DIST$
	Density (ln)	Bare Ground	$0.76 - 0.31 \times DIST$
	$r^2aj = 0.17$	Microsite	$0.76 - 0.31 \times DIST$
spiders			
	Species Richness (ln)	Bare Ground	$0.17 - 0.10 \times DIST$
	$r^2aj = 0.07$	Microsite	$0.17 - 0.10 \times DIST$
	Density (ln)	Bare Ground	$0.17 - 0.10 \times DIST$
	$r^2aj = 0.07$	Microsite	$0.17 - 0.10 \times DIST$
centipedes			
	Species Richness (ln)	Bare Ground	$0.06 - 0.11 \times ED$
	$r^2aj = 0.23$	Microsite	$0.23 + 0.14 \times ED$
	Density (ln)	Bare Ground	$0.07 - 0.04 \times AREA - 0.12 \times ED$
	$r^2aj = 0.24$	Microsite	$0.23 + 0.18 \times AREA + 0.22 \times ED$
millipedes			
	Species Richness (ln)	Bare Ground	$0.17 + 0.1 \times WOOD$
	$r^2aj = 0.23$	Microsite	$0.17 + 0.1 \times WOOD$
	Density (ln)	Bare Ground	$0.16 - 0.01 \times DIST + 0.07 \times WOOD$
	$r^2aj = 0.15$	Microsite	$0.16 - 0.01 \times DIST + 0.07 \times WOOD$
All together			
	Species Richness (ln)	Bare Ground	$1.57 - 0.48 \times DIST + 0.17 \times AREA - 0.29 \times ED$
	$r^2aj = 0.68$	Microsite	$1.94 - 0.48 \times DIST + 0.17 \times AREA + 0.01 \times ED$
	Density (ln)	Bare Ground	$2.18 - 0.60 \times DIST + 0.45 \times LPI - 0.19 \times ED$
	$r^2aj = 0.69$	Microsite	$3.15 - 0.60 \times DIST + 0.45 \times LPI + 0.33 \times ED$

density and species richness and to centipedes density in microsites only. Finally, patch density (PD) was the least influential variable, and was negatively correlated to earthworm species richness and density.

3.3. Relationships between soil temperature and water content and vegetation cover

The presence of grass tufts had a significant effect on soil temperature in the upper 15 cm of the soil (Fig. 2a), where soil macrofauna density was also highest (Fig. 2b). There was a difference of 5 °C between the soil, in upper 5 cm, below the centre of the tuft (29 °C) and the hottest location in bare ground (34 °C), Horizontal maps confirmed this result and showed that soil temperature was strongly dependent on the distance to the nearest grass tuft (white points in Fig. 3a). Within the grass tufts, soil temperature increased from the centre to the edge of the tuft, varying from 28 °C to 30 °C (black points in Fig. 3a). However, there was no significant relationship between the water content and the distance to the edge of the nearest grass tuft (Fig. 3b). Table 3 shows the variogram parameter for both soil temperature and water content measured in the different sampling grids. A spherical model satisfactorily fitted the variograms observed in each case. The variogram parameters changed depending on plot size and the minimum inter-sample distance. The range, sill and nugget variance tended to increase with increasing map size (Table 3). There was remarkably little unexplained variation in soil temperature since nugget variance which ranged from 3.4% to 6.4% depending on the plot size (Table 3). However, nugget variance was high for soil water content, ranging from 40% to 37% for plots of 1 and 9 m^2 (Table 3). Both data sets showed that the range was smaller than one third of the plot length.

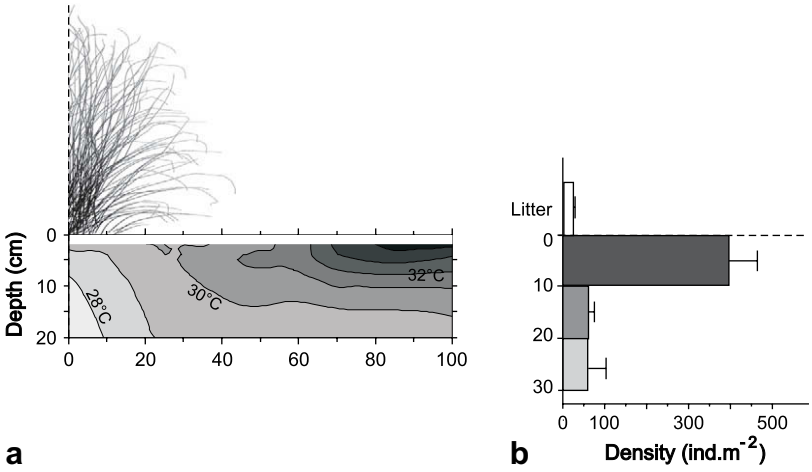

Fig. 2. a) Vertical profile of soil temperature below and near a grass tuft. b) Vertical profile of soil macrofauna density in the upper 30 cm of soil ($n = 60$).

The isarithmic maps for the 9 m^2 plot were obtained by ordinary kriging with the variogram parameters shown in Table 3. Because the variogram range was low, the temperature map showed small patches of high values (Fig. 4a). The high temperature areas were usually located between grass tufts while low temperature areas were located beneath the tufts (Fig. 4a). The map of soil water content showed larger patches of high values compared to soil temperature (the variograms showed larger ranges, Table 3, Fig. 4b) and there was no clear relationship with tuft distribution and temperature.

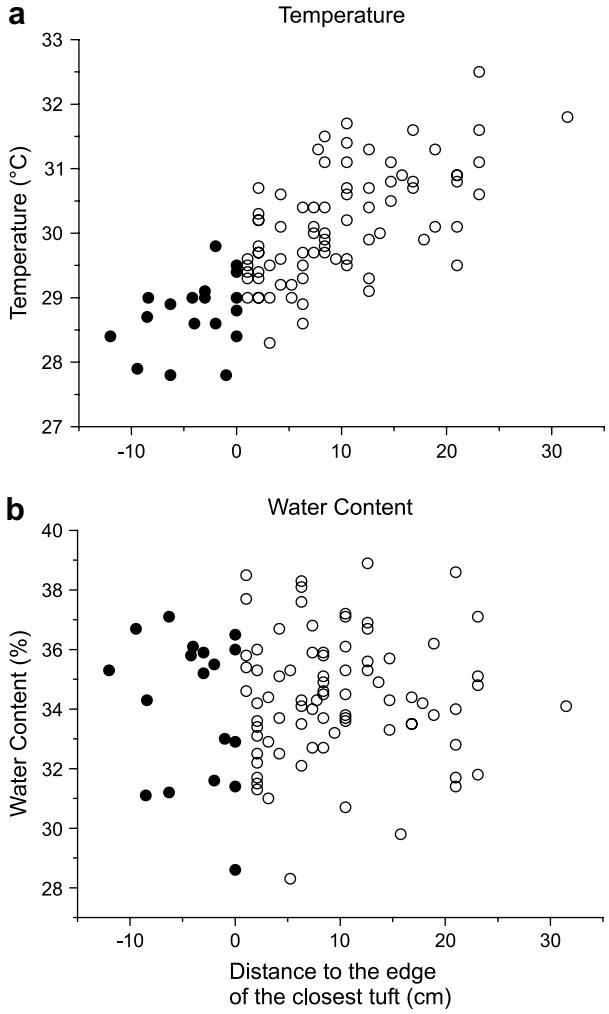

Fig. 3. Relationship between the distance to the edge of the nearest grass tuft and a) the soil temperature, and b) the soil water content, in the 9 m^2 map. Black points represent measures taken inside the grass tufts, white points indicate measures taken outside the grass tufts.

4. Discussion

Our survey showed that the spatial structure of the vegetation cover affected both soil macrofauna density and species richness. This has been well documented for surface invertebrates (Hatley and Macmahon, 1980; Hamazaki, 1996), but data on soil living organisms are less common.

4.1. Defining mechanisms scale

In pastures, the factors affecting soil macrofaunal communities can be considered at two scales: (i) the micro-site scale, where the only factor of interest is the nature of the sample (bare ground, or microsite) and (ii) the "micro-landscape" scale, where the environment surrounding the sample is also taken into account to explain the soil macrofauna biodiversity

4.2. Micro-site scale effects

Micro-site scale effects were straightforward: samples taken below herb tufts or branches hosted a much higher abundance and diversity of soil macrofauna than the bare ground, showing a striking local limitation by habitat and/or food availability. For instance, a dead trunk on the ground was seen to be a specific resource that favored diplopod and termite activity, especially the soil and wood feeding genus *Amitermes* (Termitinae), that was dominant in our study (data not shown). *B. bryzantha* tussocks offer both specific environmental and feeding resources for soil macrofauna and thus their size and shape influence soil macrofauna biodiversity (Mathieu

Table 3
Parameters for the models fitted to the soil temperature and soil water content semi-variograms, in the 1 m^2 and 9 m^2 maps. The range indicates the distance at which the sill was reached.

Variable	Grid extent (m)	Mesh size (m)	Model	Nugget (C_0)	Sill (C)	Range (m)
Temperature	1 × 1	0.1 × 0.1	spheric	0.02	0.57	0.34
Temperature	3 × 3	0.3 × 0.3	spheric	0.07	1.02	0.60
Water content	1 × 1	0.1 × 0.1	spheric	2.25	3.32	0.40
Water content	3 × 3	0.3 × 0.3	spheric	2.7	4.64	1.00

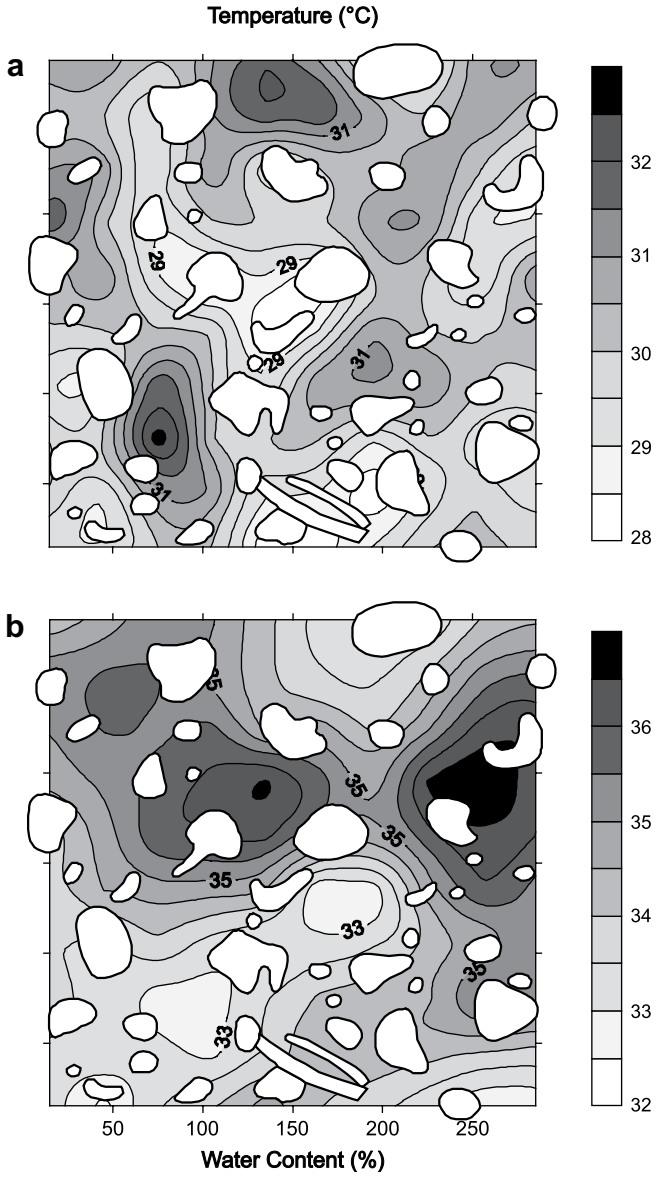

Fig. 4. Interpolated maps of the soil temperature a), and the soil water content b), on a 9 m^2 surface. Parameters from the semi-variograms (Table 3) were used for kriging. Grass tufts are shown as white surfaces delimited by a black line.

et al., 2004). Grass tussocks are therefore biodiversity hotspots for soil macrofauna in Amazonian pastures.

4.3. Micro-landscape scale effects

Nevertheless, we observed that the difference between bare ground and grass tufts is more subtle than it first appeared. Soil macrofauna biodiversity was seen to (1) decrease with increasing distance to the nearest grass tuft (DIST) (2) increase with increasing vegetation cover (AREA) (3) be influenced by the size of the largest herb tuft in the micro-landscape (LPI). These remote effects appear to be due to *B. bryzantha* gradually inducing modifications to the surrounding environment. Indeed, plants are known to change their micro-environments by intercepting sun rays and rain and absorbing soil water (Geiger and Aron, 2003). In addition, they modify the chemical properties of the soil near their roots by adsorbing mineral nutrients and releasing organic-C exudates, lowering pH, activating microflora, and deposing litter (Jackson and Caldwell, 1993; Amiotti et al., 2000). This process known as "ecological engineering" (Jones et al., 1994) creates a gradient of specific physical and chemical properties which are beneficial to the soil microflora (Zaman and Chang, 2004), and also probably to soil macrofauna, mainly through bottom-up processes such as increasing soil organic matter. *B. bryzantha* grasses also influenced the soil environment by cooling down and reducing soil temperature variations beneath and around them, in the upper 15 cm of soil, where soil macrofauna is the most abundant. Numerous studies have shown that soil macrofauna in tropical areas is limited by high temperatures (earthworms: Uvarov and Scheu, 2004; Opilions: Almeida-Neto et al., 2006; ants: Albrecht and Gotelli, 2001; termites: Smith and Rust, 1994; coleoptera: Horgan, 2002) and that temperature is a strong determinant of many soil macrofauna ecological niches (Bezkorovainaya and Yashikhin, 2003). Thus, the reduction of soil temperature observed here due to the *B. bryzantha* tussocks is likely to have important effects on soil macrofauna, at least during the day.

Nevertheless, we suggest that micro-landscape scale effects not only result from the modification of the environment in the vicinity of the tufts. These also appear to exist because of limitation by habitat and/or resource availability for species with homing range larger than just the size of our samples. For instance, the observed increase in ant density with increasing vegetation cover (AREA), as well as the increase in termite abundance with the size of the largest herb tuft (LPI) may be explained by the fact that the galleries and chambers produced as part of their nest-structures are preferably constructed below herb tufts and are organized in networks with connections to other grasses (Mathieu et al., 2004). As a consequence, a remote increase of habitat availability or suitability can lead to local increases in the density of the colony due to the interconnections between chambers, while loose vegetation cover may lead to habitat and/or resource-limitation.

4.4. Movement patterns

Lastly, micro-landscape effects may occur by modifying movement patterns of individuals. Such effects were previously demonstrated for surface beetles which followed different foraging trajectories depending on the micro-landscape configuration on a 25 m^2 scale (Wiens and Milne, 1989). In our study, connectivity, measured by the edge density (ED), and patch density (PD) (Giles and Trani, 1999) was related to soil macrofauna biodiversity. Theoretically, if assuming that soil fauna movements are random, a longer edge will increase the probability of encountering the habitat. It was shown experimentally that higher numbers of millipedes inhabited patches with long edges than other patches with the same area but shorter edges (Hamazaki, 1996). However, it is doubtful that this phenomenon can be transposed to the whole soil macrofauna community. In particular, soil fauna movements are not necessarily random and information is required on the range of daily movements made by the different groups. With the exception of species that construct nests, there is currently little information available regarding foraging behavior among the groups found and the distances they are able to cover daily. Social insects (ants and termites) create costly perennial nest-structures that require foraging on scales much larger than 9 m^2. Although foraging efficiency may be influenced by habitat connectivity, it is unlikely that it constitutes a limiting factor for social insects. Higher vegetation cover may also favor movement by motile organisms such as millipedes because it provides shelter from predators, sunlight and high temperatures. Therefore in dense vegetation cover, organisms can extend their foraging range at a low cost. To confirm this hypothesis, it would be interesting to study soil macrofauna movement amid different micro-landscape configurations,

using a technique such as individual tagging for example (e.g., Butt and Lowe, 2007).

4.5. Reversing the correlations: feedback loops between plants and soil engineers

Interestingly, the density and species richness of earthworms, termites and ants showed the best correlations with the vegetation pattern. Since all of these animals are soil ecosystem engineers, they are assumed to induce positive feedback loops on vegetation growth (Jouquet et al., 2006). Therefore, the correlation between their abundance and vegetation cover or the area of largest grass tuft could be due to improved plants growth in the presence of soil engineers. Because increased vegetation cover is then also beneficial to soil macrofauna, grass tufts and soil macrofauna appear to be involved in a reciprocal beneficial relationship.

4.6. Conclusions

Our study shows that *B. bryzantha* tufts have a strong influence upon soil macrofauna diversity and abundance within pasture ecosystems at both the micro-site ($\leq$0.016 m^2) and micro-landscape (9 m^2) scales. These environments provide habitats and create complex gradients of soil properties to which soil macrofauna respond. Therefore to fully understand soil macrofauna biodiversity distribution in these systems a careful study of the vegetation cover around the samples is required. We argue that these types of patterns are not unique to Amazonian pastures, but are also likely to occur in many other systems and should be taken into account in soil macrofauna biodiversity studies.

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Annexe 7

Caro, G., T. Decaëns, C. Lecarpentier, and J. Mathieu, Are dispersal behaviours of earthworms related to their functional group? Soil Biology and Biochemistry, 2013. 58(0): p. 181-187.

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Are dispersal behaviours of earthworms related to their functional group?

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ABSTRACT

Dispersal plays a key role in the dynamics of ecological communities as it strongly determines the potential of individuals to colonize new habitats. Understanding and predicting species dispersal behaviour is therefore central to any effort at managing or even understanding the formation of communities. In this context, it is essential to understand the influence of environmental and biotic determinants of dispersal. In this work, we assessed these questions using earthworms as model organisms.

We assessed the dispersal behaviour of six earthworm species belonging to two different functional groups (i.e. three anecics and three endogeics) in response to three key environmental factors: habitat quality, intraspecific density, and environment homogeneity. We found that habitat quality significantly influenced the dispersal rates of all species. Intraspecific density increased the dispersal rate of the three anecic species but only of one endogeic species. In a homogeneous environment, anecics dispersed further and in greater proportion than the majority of endogeics. Moreover, few anecic species have shown a tendency to follow conspecifics. Overall, anecic species seemed to have a higher active dispersal inclination than most endogeic ones. We found a high variability of our results within each functional groups, which suggests that this classification cannot be used to explain or predict the dispersal behaviour of earthworms.

1. Introduction

The link between biodiversity and ecosystem functioning is a central issue in ecology (Loreau et al., 2001; Duffy, 2002; Hooper et al., 2005). Previous studies pointed out the richness of functional groups – groups of species with similar functional traits (Blaum et al., 2011) – as being of particular importance for ecosystem functioning (Hector et al., 1999; Schwartz et al., 2000). Moreover, the mobility of animal species may result in complex relationships between functional group richness and ecosystem functioning. Predicting the spatial distribution of individuals hence appears as a requirement to manage populations of ecosystem engineers, in order to maintain the ecosystem services they deliver (Clobert et al., 2001; Petchey and Gaston, 2002). Given the variety of animals, it is necessary to search for general rules that predict their spatial distribution. A central point to address in this issue is the magnitude of the link between functional groups and dispersal behaviours.

Dispersal is a central ecological process that allows colonization of new habitats and exploitation of spatially and temporally variable resources (Ronce, 2007). Active dispersal of animals (opposed to passive dispersal, where individuals could be transported by an external agent and has not necessarily a cost for individual) is the result of three successive behavioural stages (following the definition given by Clobert et al., 2001, 2009). It involves the departure from a breeding site, crossing to a new place, and settlement. It can occur at any life stage, at any spatial scales above the individual range and within more or less heterogeneous landscapes (Clobert et al., 2009). It is assumed to depend on the balance between the costs and benefits of dispersal (Bowler and Benton, 2005; Bonte et al., 2012), which are strongly determined by both environmental conditions (e.g. habitat quality, habitat fragmentation, patch size, density, predation) and individual life traits (e.g. age, hormonal levels; (Bonte et al., 2006; Schtickzelle et al., 2006)). Ecosystem engineers, such as earthworms, are species that can modify physically their surrounding environment in a specific way (Jones et al., 1994). These modifications could therefore interact with population density and drive complex dispersal behaviours. As species belonging to a given functional groups are expected to modify their environment in a similar way, it could also be expected to find a concordance between functional classification and dispersal behaviours.

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Earthworms provide a good model to assess the concordance between functional groups and dispersal behaviours. Indeed, earthworm species can be classified in contrasted groups, based on their traits, ecology and functional role regarding soil processes (Bouché, 1972, 1977). Besides, earthworms are of primary importance for ecosystem functioning as they modify the availability of resources for other organisms through physical and chemical changes in their surrounding soil environment (Jones et al., 1994, 2010; Lavelle et al., 2006). The most used functional classification of earthworms (Bouché, 1972, 1977) distinguishes epigeics, anecics and endogeics, based on morphological (e.g. size and pigmentation) and ecological features (soil stratum where individuals are active, food diet). Earthworm ecological preferences and impact on the environment have been largely documented (Bohlen and Edwards, 1995; Brown, 1995; Blanchart et al., 1999), as well as their burrowing behaviour (Capowiez, 2000; Bastardie et al., 2003). However the diversity of their dispersal features has been overlooked and still needs to be documented for most species and functional groups (Mathieu et al., 2010).

In this work, we tested the correspondence between earthworm functional groups and dispersal behaviours. We experimentally compared the dispersal behaviour of six species belonging to the functional groups anecics and endogeics, which are believed to have the strongest impact on soil functioning (Bouché, 1972, 1977). For each species, we tested the impact of three factors assumed to be important drivers of animal dispersal: habitat quality, conspecific density, and also the dispersal patterns in homogenous environment.

2. Materials and methods

2.1. Biological models

We used three endogeic species – *Aporrectodea icterica* (Savigny 1826), *Aporrectodea caliginosa* (Savigny 1826) and *Allolobophora chlorotica* (Savigny 1826) – and three anecic species – *Aporrectodea giardi* (Ribaucourt, 1901), *Aporrectodea longa* (Ude 1886) and *Lumbricus terrestris* (Linnaeus 1758) – which are all usually well represented in natural assemblages in North-Western France (Decaëns et al., 2008). Earthworms were collected in a forest in North-Western France (49°27′N, 1°4′E) and were reared in suitable soil (see Section 2.2) at low density (1.5 individuals per litre of soil, according to Mathieu et al. (2010), at 15 °C during the day and 10 °C at night. All individuals were used only once and were adult during the experiments.

2.2. Soils

Two types of soil were used for the experiments: an unsuitable and a suitable soil. The suitable soil (Table 1) was sampled in a grassland of the IRD research centre (48°54′E, 2°29′N) which hosts large earthworm populations. The unsuitable soil consisted of a very sandy soil with low pH (Table 1) collected in an area deprived of earthworms in the forest of Fontainebleau (48°24′N, 2°44′E). The suitable soil was from a brunisol and the unsuitable soil from a luvisol (based on the world reference base for soils, FAO). Both soils were air dried, sieved at 2 mm and rewetted manually at 25% of humidity (on a massic basis – soil water content was set by drying the soil at 105 °C during 48 h).

2.3. Experiments

We used separate standardized experimental devices (mesocosms) to study the influence of three different environmental factors on dispersal behaviours: (1) Intraspecific Density (ID); (2) Habitat Quality (HQ); (3) Homogenous Environment (HE). These treatments were chosen to address three key mechanisms shaping the spatial distribution of populations: intraspecific competition, habitat choice and spread capabilities.

Table 1
Selected properties of the soil substrate used in the experiments.

Soil properties	Unsuitable soil	Suitable soil	Unit
Clay	4.7	15.7	%
Silt	18.5	13.4	%
Sand	76.8	70.9	%
Organic C	8.5	28.1	g kg^{-1}
Total N	0.33	2.61	g kg^{-1}
C:N	25.8	10.8	
Organic matter	14.6	48.6	g kg^{-1}
pH	3.8	7.5	
CEC (Metson)	2.9	11.7	cmol kg^{-1}

The influence of intraspecific density on dispersal (Experiment ID) was studied in mesocosms that consisted of a dispersal corridor of 100 cm long, 20 cm wide and 20 cm high (Mathieu et al., 2010), which was composed of three equal sections (Fig. 1): (1) the "inoculation" section, which was filled with suitable soil; (2) the intermediate "crossing" section, composed of unsuitable soil; (3) the "arrival" section composed of suitable soil. Soil densities were 1 ± 0.1 g cm^{-3} in each section of the mesocosm. This setup allows to reproduce the three stages of dispersal: departure, crossing and settlement in a suitable site (Clobert et al., 2009). In this context, we consider that the rates of individuals leaving to the inoculation section are dispersal rates. Moreover, the unsuitable soil in the crossing section was designed to represent a physical barrier generating dispersal costs. It allowed distinguishing between mechanisms of diffusion (random movements with potentially returns in the starting point) from active dispersal (Clobert et al., 2009) and to prevent to U-turns movements (Caro et al., 2012). To assess the effect of intraspecific density on dispersal rate, we inoculated earthworms at four densities (i.e. the numbers of worms inoculated in the first section): 1, 10, 20 or 30 individuals of the same species in the ID experiment. We ensured that all individuals were inoculated in the first section by waiting that each individual entered in the soil, which took on average 10 min after inoculation.

To study the influence of habitat quality on dispersal (Experiment HQ), we used the same mesocosm design with unsuitable soil in the inoculation section (Fig. 1). For each species, we inoculated 10 individuals. Both experiments (ID & HQ) lasted seven days and were replicated 5 times under the same conditions of temperature (15 °C during the day and 10 °C at night) and light as breeding. At the end of the experiment, we counted all individuals in each section.

In the HE experiment (dispersal in a homogeneously suitable environment), we observed the dispersal in larger mesocosms of 300 cm long, 20 cm wide and 20 cm high (Fig. 1), filled exclusively with suitable soil at a bulk density of 1 ± 0.1 g cm^{-3}. This allowed documenting the natural spread of individuals while removing the effect of heterogeneity. In order to identify the location of individuals in the mesocosms, we defined 13 regular sections of 23 cm long, which we named according to their distance to the central section. For each species, 10 individuals were inoculated in the central section (i.e. section 0) at the beginning of each experiment. We limited the time of each experiment to 24 h in order to prevent U-turns by individuals reaching the end of the mesocosm. Each experiment was replicated 4 times under the same conditions of temperature and light as for breeding. At the end of the experiment, we counted all individuals in each section.

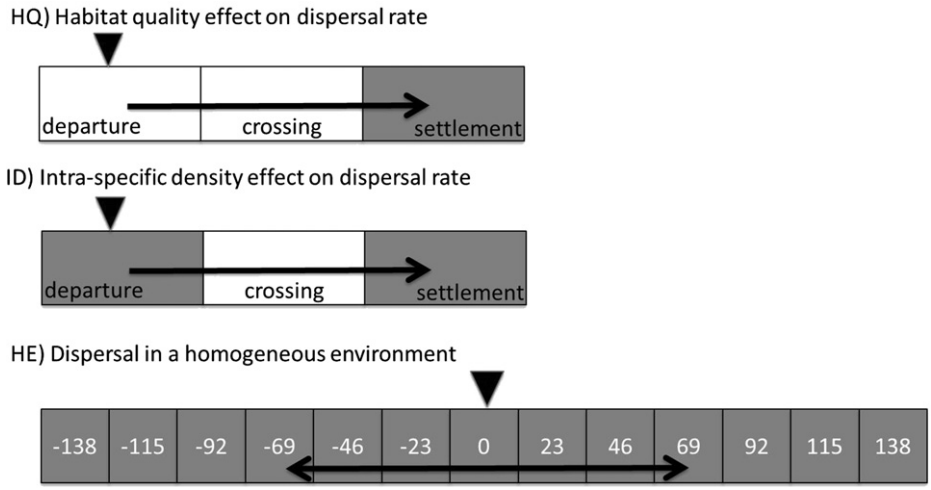

Fig. 1. Experimental designs of the dispersal studies: HQ) Habitat Quality effect on dispersal rate; ID) Intraspecific Density effect on dispersal rate; HE) dispersal in a Homogeneous Environment; White area = unsuitable soil; Grey area = suitable soil; triangles = exact location where earthworms were introduced (inoculation points).

In order to identify the influence of the body size on the dispersal responses, all individuals were weighed individually before and after each experiment. We found no significant effect of individual biomass on the dispersal behaviour (p-value > 0.05, Linear Model), and we also found that body size did not change significantly between the beginning and the end of the experiments (p-value > 0.05, Linear Model). In consequence, body size was not considered in further statistical analyses.

2.4. Dispersal quantification and statistical analyses

In ID and HQ experiments, we defined the dispersal rate as the proportion of individuals that moved from the inoculation section to the third section. For the ID experiment, we used a General Linear Model (GLM) with a binomial family to compare the dispersal rates at each density level.

To test differences in dispersal responses to the density increasing among species, we fitted non-linear models on the dispersal rates in response to increasing intraspecific density:

$$D_k(d) = \mathrm{IDr}_k * d^2 / \left(\mathrm{IDs}_k^2 + d^2\right)$$

where:

- $D_k(d)$ represent the dispersal rate of species k at density d;
- d represents the intraspecific density of the species;
- IDr_k represents the value at which the model reaches a plateau. It allows us to know the maximum dispersal rate of the species k in response to the intraspecific density increasing;
- IDs_k represents the inflection point of the model. It informs us on the density sensitivity of the species k.

We further used the estimated parameters, IDr_k and IDs_k, to perform pair-wise comparisons between functional groups with a Linear Model.

For the HQ experiment, we tested the influence of habitat quality on dispersal by comparing the observed dispersal rates to those obtained from a suitable environment, i.e. the ID experiment at a density of 10 individuals. Differences were tested using a GLM with a binomial family. To quantify earthworm sensitivity to habitat quality, we calculated the percentage of difference (HQs) between these two dispersal rates for each species: $\mathbf{HQs} = (\boldsymbol{D}_{\mathbf{unsuit},k} - \boldsymbol{D}_{\mathbf{suit},k})/\boldsymbol{D}_{\mathbf{unsuit},k} * \mathbf{100}$, where D_k represents the dispersal rate from an unsuitable (unsuit) or a suitable (suit) environment of the species k. In this way, the HQs varies from 0 to 100 %; the HQs maximum value meaning that the dispersal of species k is almost only triggered by habitat quality. We used this index to compare the sensitivity of both functional groups to habitat quality with a Linear Model.

In the HE experiment, dispersal rate (HEr_k) was calculated as the proportion of individuals of a species k that left the central section (position 0; Fig. 1). We also computed the average distance crossed for each species (HEd_k) and a distribution index: $\mathbf{HEf}_k = \mathbf{|(HEr}_k \textbf{ left} - \mathbf{HEr}_k \textbf{ right)|}$, where: HEr_k represents the dispersal rate at left or at right of starting section; Thus, HEf_k varies from 0 (individuals equally distributed in each side) to 100 (all individuals in only one side) and gives an idea of the proportion of individuals that followed each other – the more HEf_k is high, the more the species k have a high tendency in following conspecifics. We did not use a classic index of asymmetry because of boundary effects related to the mesocosms. In order to compare the species and functional group dispersal capabilities, we compared the mean distance crossed (HEd), the mean dispersal rates (HEr) and the tendency to follow conspecifics (HEf) with a Linear Model.

2.5. Dispersal behaviours synthesis

In order to synthesize these results, we constructed a table with the different species dispersal features: IDr_k (maximum dispersal rate in response to intraspecific density), IDs_k (sensitivity to intraspecifics density), HQs_k (sensitivity to habitat quality), the HEd_k (mean distance crossed in the homogeneous environment), HEr_k (mean dispersal rate in the homogeneous environment) and HEf_k (average tendency to follow conspecifics). Afterwards, we performed a Principal Component Analysis (PCA) on the centred and standardised table. This allowed depicting synthetically the link between ecological categories and dispersal behaviours. We retained 2 axes in the PCA, which accounted for 94.5% of the total inertia. To represent the dispersal strategy of each species, we plotted species on the PCA space with segment diagrams where each segment represents a dispersal parameter and the size of the segment represents the value of the parameter. This representation is similar to the standard correlation circle. All analyses were performed with the ADE-4 package from R (Ihaka and Gentleman, 1996; Thioulouse et al., 1997).

3. Results

3.1. Dispersal behaviours in detail

Intraspecific density was determinant in dispersal behaviours, except for *A. caliginosa* and *A. chlorotica*. For the other four species, when the density reached the threshold of 10 individuals, dispersal rates increased significantly (within each species, p-value < 0.05, binomial GLM; Fig. 2). Above the density of 10 individuals, the dispersal of *A. longa* and *L. terrestris* did not change significantly (within each species, p-value > 0.05, binomial GLM) whereas the dispersal of *A. icterica* and *A. giardi* significantly increased at 30 individuals per section (p-value < 0.05, binomial GLM; Fig. 2). Non-linear regressions showed that the sensitivity to intraspecific density was not strongly related to the dispersal threshold (here 10 and 30 individuals). For instance, *A. longa* had a value of IDs ten times lower than that of *A. giardi*. Although statistical analysis did not show any significant difference in the dispersal parameters among functional groups (IDr and IDs not significantly different), we observed that all anecics were density sensitive while among endogeics only *A. icterica* dispersed more at higher densities.

Dispersal rate of earthworms inoculated in the unsuitable soil was in average 83% higher than in the suitable soil (within each species, p-value < 0.01, binomial GLM; Fig. 3). The most sensitive species to habitat quality were the two endogeics *A. chlorotica* and *A. caliginosa*, with HQs of 100% for each of them. Anecics were also sensitive to habitat quality, with HQs $= +83 \pm 8.5\%$ in average. We found no significant differences in HQs among the two functional groups (p-value > 0.05, Linear Model).

In the homogeneous environment, all species dispersed from the inoculation section (Fig. 4). *A. icterica* moved significantly more than other species, either regarding the proportion of individuals that had dispersed (HEr), or the average distance crossed (HEd). Anecics dispersed significantly more than the two others endogeics. Finally, the index HEf suggested that the dispersal direction of *A. giardi* was influenced by the previous passage of congeners ($HEf_{giardi} = 74.8$) while *A. icterica* and *A. longa* seemed to avoid conspecifics ($HEf_{icterica} = 16.1$ and $HEf_{longa} = 19.7$) (Fig. 4).

3.2. Synthesis of dispersal behaviours

The first axis of the PCA accounted for 67.07% of the total variance and discriminated *A. chlorotica* and *A. caliginosa* (positive scores) from *A. icterica* (negative score; Fig. 5). It was highly associated with the maximum dispersal rate in response to intraspecific density (IDr), average distance crossed (HEd) and average dispersal rate (HEr) in a homogeneous environment, and, to a lesser extent, to the sensitivity to habitat quality (HQs) (Fig. 5). It was thus interpreted as a gradient in endogeics dispersal capabilities. The second axis accounted for 27.43% of the total variance and discriminated *L. terrestris* and *A. longa* (positive scores) from *A. giardi* (negative score) (Fig. 5). It was associated to the sensitivity to intraspecific density (IDs) and to the tendency to follow conspecifics (HEf) (Fig. 5) and was therefore interpreted as the influence of conspecifics on dispersal of anecic species.

4. Discussion

We observed that the distribution of endogeic species on the PCA plan can be explained by differences in dispersal capabilities, such as the distance crossed and the dispersal rate (Fig. 5). In this analysis, *A. icterica* had the highest dispersal basal rate among endogeics, while the other two species of this functional group only dispersed in response to strong environmental stimuli, such as unsuitable soil conditions (Figs. 2 and 3). The anecic species distribution on the PCA plan appeared to be strongly defined by differences in their sensitivity to conspecifics: they were the most sensitive species to intraspecific density (with a very low IDs value;

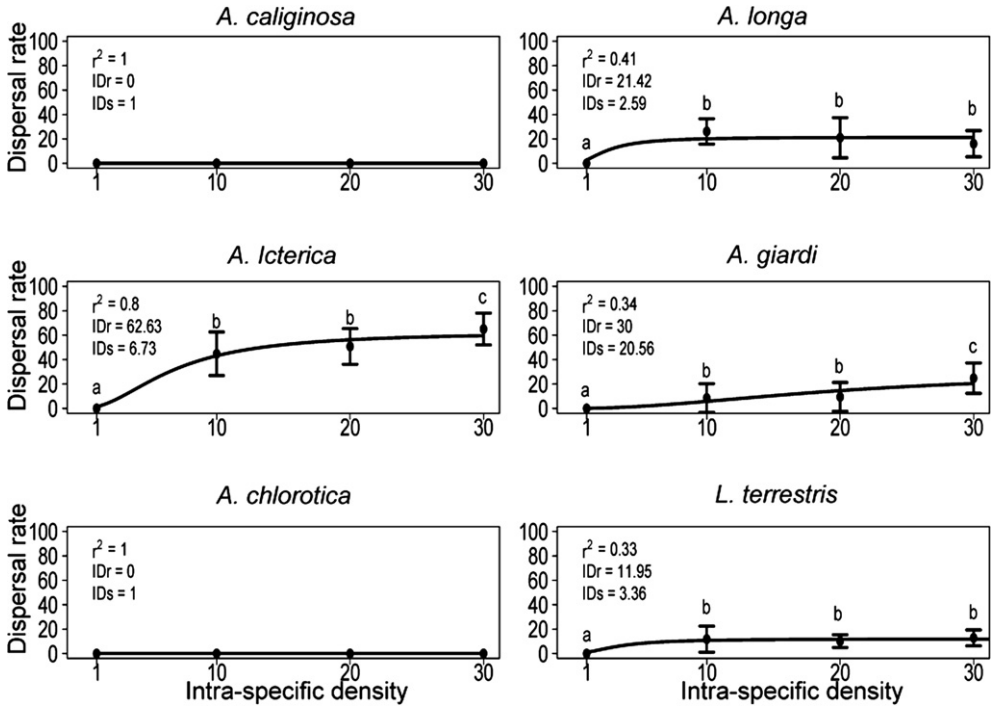

Fig. 2. Dispersal rate in response to the intraspecific density (mean ± standard deviation; $N = 5$). Different letters indicate significant differences at $p = 0.05$ (General Linear Models with a binomial family). The line represents the non-linear regression by fitting the equation $D_k(d) = IDr_k * d^2/(IDs_k^2 + d^2)$ on the dispersal data of the species k, where d represent the intraspecific density and $D(d)_k$, the dispersal rates of the species k. The IDr_k and IDs_k values represent the parameters of this function and the r_k^2 is the coefficient of determination between the curve fitted and the data from the species k.

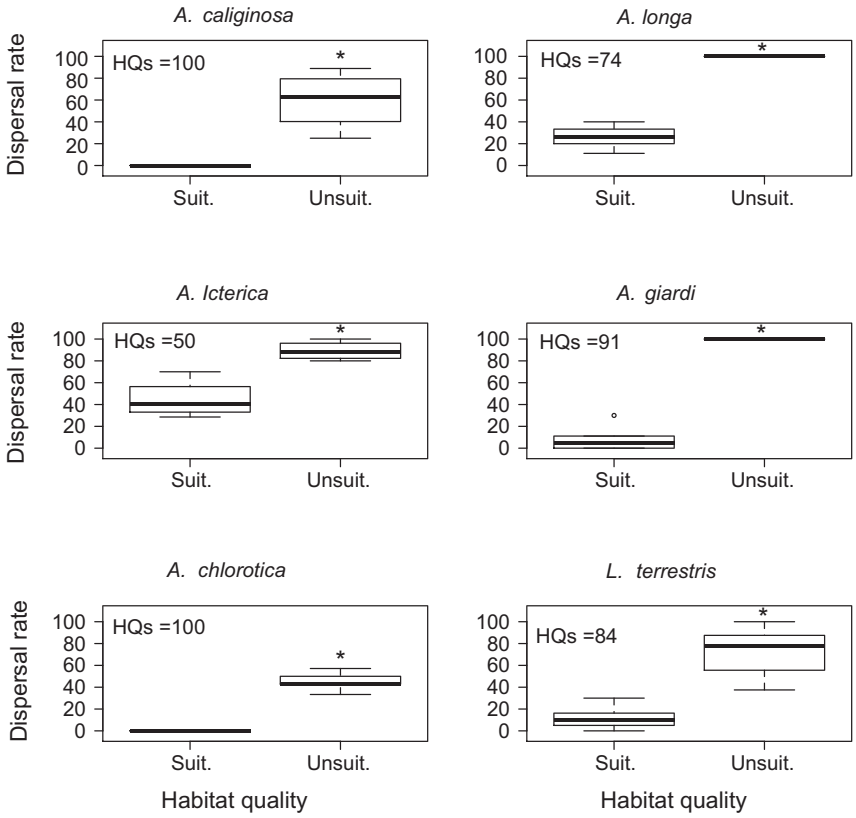

Fig. 3. Boxplot of dispersal rates in response to the habitat quality ($N = 5$); suit. = suitable; unsuit. = unsuitable; HQs = percentage of difference between the dispersal rates; * indicate significant differences at $p = 0.05$ (General Linear Models with Binomial response).

Fig. 2) and had a net tendency to follow their conspecifics (*L. terrestris* and *A. giardi* had an HEf index high; Fig. 4). Consequently, we did not observe any strong correspondence between earthworm functional groups and the groups of species identified by the PCA on the basis of their dispersal behaviours. This observation highlights the diversity of dispersal behaviours among species.

Some degree of functional redundancy is expected among species of a single functional group, and this implies that the disappearance of one or more of those species is not expected to affect ecosystem processes in a significant way because the remaining species can compensate for it (Naeem, 1998; Walker et al., 1999). In a context of increasing environmental disturbances, the

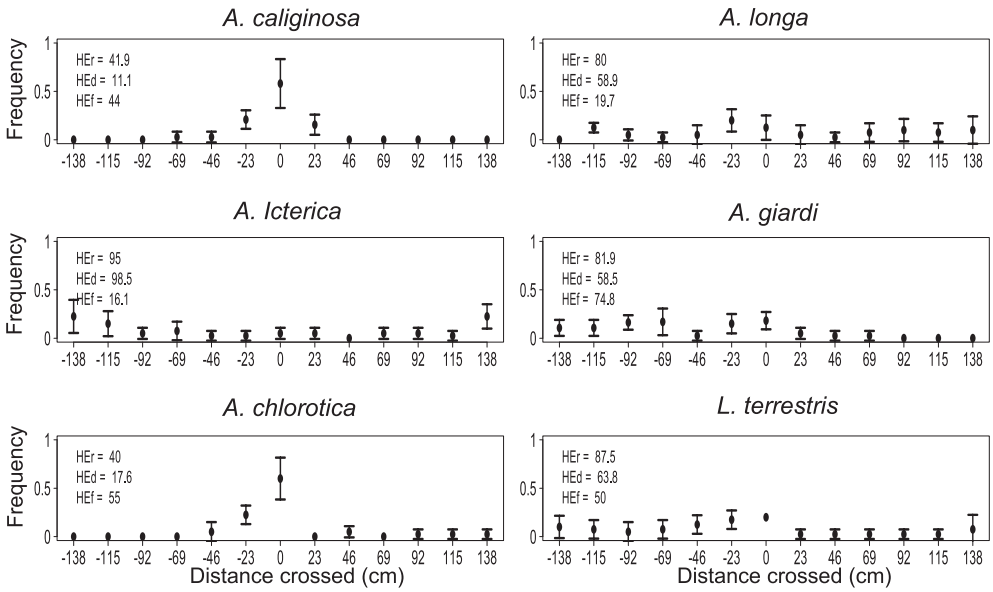

Fig. 4. Distribution of individuals according to the distance to the inoculation section ($N = 4$). We represented the mean proportion of individuals in each part of the mesocosm (mean ± standard deviation). HEr = mean dispersal rate; HEd = mean distance crossed; HEf = tendency to follow conspecifics.

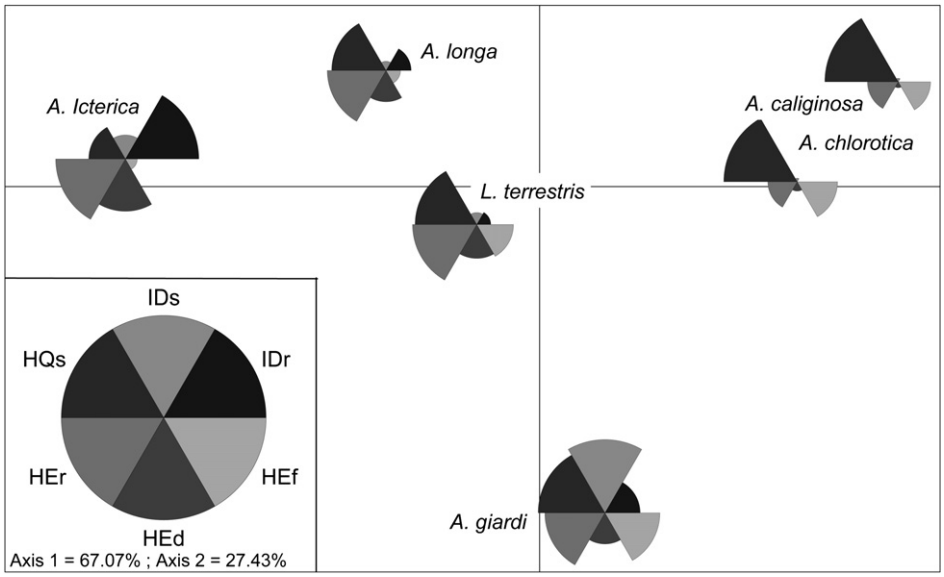

Fig. 5. Ordination of the species according to their dispersal behaviours in the plane defined by the axis 1 and 2 of the PCA. The position of the species is represented by their name associated to a segmented diagram where each segment represents a dispersal parameter and the size of the segment is proportional to the value of the parameter. IDr = maximum dispersal rate in response to the intra-specific density; IDs = sensibility to the intra-specifics density; HQs = sensitivity to the habitat quality; HEd = mean distance crossed in the homogeneous environment; HEr = mean dispersal rate in the homogeneous environment; HEf = average tendency to follow conspecifics.

presence of a pool of species responding differently to the same perturbation may allow the maintenance of the diversity of their functions. The diversity in dispersal behaviours observed in our study may for instance facilitate the maintenance of a group by different mechanisms of dispersal and different potentials for colonizing disturbed habitats (Walker, 1992, 1995; Diaz and Cabido, 2001).

Several studies reported a negative influence of high intraspecific densities on the growth rate and maturation of both endogeic and anecic earthworms (Eriksen-Hamel and Whalen, 2007; Uvarov, 2009). In addition, anecics are usually negatively impacted by endogeics, whereas the presence of anecics is often considered as beneficial to endogeics (Uvarov, 2009). From an evolutionary point of view, the fact that anecics seem negatively influenced by both intra- (based on our observations; Fig. 2) and interspecific density (Uvarov, 2009) suggests that they may have evolved greater dispersal capabilities than most endogeic species in order to minimize these negative interactions (Clobert et al., 2001). In comparison, it seems that endogeics such as *A. caliginosa* evolved weak dispersal abilities (Uvarov, 2009). Our results suggest that endogeics could have high capacities to take advantage of the burrowing activities of other species, a hypothesis supported by previous studies (Capowiez, 2000; Jégou et al., 2001; Uvarov, 2009). This could explain in part the low dispersal rates observed in *A. chlorotica* and *A. caliginosa*.

The fact that anecic species present a higher active dispersal capabilities than the majority of endogeics, in the specific context of our experiment, suggests that they could be the first colonizers of new habitats, paving the way for other species through the building of a network of re-usable galleries (Butt et al., 1999; Capowiez, 2000; Caro et al., 2012). However, this assumption is only partly supported by available observations of the dynamics of earthworm species assemblages during the colonization of new habitats. For instance, Decaëns et al. (2011) described the dynamics of earthworm communities after cropping cessation in North-Western France and identified a group of 'pioneer' species that includes two anecics but also two endogeics. In the Netherlands, Eijsackers (2011) observed that recent polder soils were at first colonized by endogeics. It is clear that our results are not directly comparable to *in situ* observations (Lee, 1985; Butt et al., 1999; Grigoropoulou and Butt, 2010), but they however suggest that earthworms have more diversified dispersal behaviours than previously assumed (Fig. 5). This highlights the importance of taking into account dispersal behaviours in studies of community assembly in new habitats or in agricultural soils where earthworms have been previously eliminated by management practices.

Despite a few similarities between species of the same functional group, we cannot deduce from our results general dispersal characteristics for each ecological category of earthworms. This is surprising because earthworm functional groups are assumed to reflect evolutionary pathways that led to the acquisition of adaptive ecological traits, and they should therefore be strongly congruent with ecological strategies evolved by species in response to e.g. predation, resource availability and/or physical constraints related to soil characteristics. Consequently, we expected species belonging to the same functional group to show similar dispersal behaviours. Alternatively, dispersal traits could be inherited from a common ancestor and in that case they should reflect the phylogenetic relationships between species. Our results do not clearly support neither the adaptive nor the phylogenetic origin of dispersal traits in earthworm species. Firstly, we found a significant degree of diversity in species dispersal behaviours within the ecological groups considered (Fig. 5). Secondly, although earthworm taxonomy is probably still not fully resolved (Decaëns et al., in press), we observed different dispersal behaviours among four species within the genus *Aporrectodea*. It therefore seems that dispersal behaviours evolved under the influence of environmental constraints that are weakly related to those that drove the evolution of functional traits, leading to the observed lack of congruence between dispersal behaviours and functional groups.

5. Conclusion

Our study highlights the diversity of dispersal behaviours among earthworm species. Considering this diversity in strategies to conserve the functional potential of earthworm communities

should be of critical importance. Indeed, pools of species with similar functional traits but responding differently to the same environmental factor should present a higher resilience when submitted to environmental disturbances. Finally, our study raises the question of the evolutionary forces (e.g. environmental disturbances, intra- and interspecific interactions) that drive the acquisition of dispersal behaviours. For instance, differences in environmental stability could lead to differences in adaptive capacity (Bonte et al., 2003; Rainio and Niemelä, 2003): a very stable environment could lead to extremely specialized species that are more likely to disperse to find optimal conditions, whereas unstable environment may lead to more generalist species.

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Annexe 8

Mathieu, J., S. Barot, M. Blouin, G. Caro, T. Decaëns, F. Dubs, L. Dupont, P. Jouquet, and P. Nai, Habitat quality, conspecific density, and habitat pre-use affect the dispersal behaviour of two earthworm species, Aporrectodea icterica and Dendrobaena veneta, in a mesocosm experiment. Soil Biology and Biochemistry, 2010. 42: p. 203-209.

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Habitat quality, conspecific density, and habitat pre-use affect the dispersal behaviour of two earthworm species, *Aporrectodea icterica* and *Dendrobaena veneta*, in a mesocosm experiment

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ABSTRACT

Dispersal capacity is a life-history trait that may have profound consequences for earthworm populations: it influences population dynamics, species persistence and distribution and community structure. It also determines the level of gene flow between populations and affects processes such as local adaptation, speciation and the evolution of life-history traits. It may play a great role in soil functioning by determining the spatial distribution of ecosystem engineers such as earthworms. Dispersal is an evolutionary outcome of the behaviour in response to the ecological constraints of the species. Hence different dispersal behaviour is expected from the different ecological types of earthworms. Nevertheless the dispersal behaviour of earthworms has been little documented.

In this work we test a series of basic mechanisms that are fundamental and complementary to understand earthworms dispersal behaviour. We focus on the dispersal triggered by environmental conditions, a fundamental process usually termed "conditional dependent dispersal". We show experimentally in mesocosms that in one week: 1) earthworm dispersal can be triggered by low habitat quality, either through soil quality or the presence/absence of litter. 2) Earthworms can be subject to positive density dependent dispersal, that is the rate of dispersal increases when density increases; and 3) earthworm dispersal can be reduced by the pre-use of the soil by conspecific individuals that are no longer present.

Our results suggest that earthworms may be more mobile than expected from previous estimations, and that they present high capacities of habitat selection. In the light of our findings we elaborate a behavioural scenario of earthworm foraging, and propose several priority working directions.

1. Introduction

Dispersal is a central ecological process that has overwhelmingly important implications at multiple organization scales (Clobert et al., 2001). It directly affects the dynamics and persistence of populations, the distribution and abundance of species, the structure of natural communities and may influence ecosystem functioning through movements of keystone species and/or ecological engineers (Cuddington and Hastings, 2004). It is therefore a key parameter to explain species distribution from a local to a biogeographical scale (Hengeveld and Hemerik, 2002). As a consequence, the study of dispersal has become a major field in ecology (Nathan, 2003). Because of the direct relationship between dispersal behaviour and fitness, a wealth of literature focused on the evolution and consequences of dispersal capacity, mainly in the framework of Optimal Foraging (Charnov, 1976), of the Ideal Free Distribution (Fretwell and Lucas, 1970; Krivan et al., 2008), of the Metapopulation Theory (Hanski and Gilpin, 1997), and of the Metacommunity theory (Holyolak et al., 2005). A central point that emerges in all these works is the necessity to determine the conditions that induce dispersal behaviours. They have been described for a large body of organisms, especially the easily sampled ones such as plants, birds, insects and fishes (Nathan, 2001). Some fundamental factors seem to operate on all organisms,

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such as habitat quality and population density. However it was also pointed out that specific dispersal mechanisms evolved among some taxa as products of particular ecological conditions. Consequently specific studies are required to understand properly the movements of any target taxonomical group.

Soil organisms face very specific ecological conditions compared to aboveground and aquatic organisms which have been the biological models for most dispersal studies (Nathan, 2001). They likely evolved original dispersal strategies due to the solidity, opacity and high spatio-temporal heterogeneity of the soil as well as the low energetic value of the soil organic matter they feed on. Despite these specificities, very few studies focused on underground soil fauna dispersal behaviour. For instance, although earthworms play a critical role in soil and ecosystem functioning (Lavelle and Spain, 2001), their dispersal behaviour still remain little investigated.

Studying earthworm dispersal would probably bring interesting new insights into the general framework of dispersal ecology. For instance, in a set of related species with contrasted ecology (such as endogeics, anecics and epigeics for earthworms), different dispersal behaviour is expected according to the ecological type of the species, but this point has not been addressed. In addition, it would provide basic information to improve field techniques of earthworm inoculation that aim to restore soils and increase crop production (e.g. Senapati et al., 1999). It would also help to explain the patterns of earthworms invasions in several regions (Tiunov et al., 2006). Finally, earthworms are potential dispersal vectors for parasites (Field and Michiels, 2006), plant and human pathogens (Toyota and Kimura, 1994; Williams et al., 2006), nematodes (Shapiro et al., 1995), ectomyccorhizal fungi (Reddell and Spain, 1991) and viable plant seeds (Decaëns et al., 2003). Therefore earthworm dispersal may have numerous consequences in agriculture and ecosystem functioning.

In this work we present the results of an experimental study that focused on three mechanisms that might lead to earthworm active dispersal. First we assessed whether habitat quality (soil properties or presence of litter on soil) may trigger earthworm dispersal. Many previous works on other organisms showed that low habitat quality generally induces active dispersal. Low resource availability, for instance, is known to increase intra-specific competition (Balkau and Feldman, 1973) of many groups. In a second step, we considered the role of earthworm density on their dispersal. Previous works showed that most terrestrial animals are prone to positive density dispersal (that is dispersal rate increases with increasing density, Matthysen, 2005). Nevertheless some species do not present this trend (Midtgaard, 1999; Bodasing et al., 2001) and some even show negative density dependence dispersal, i.e. a tendency to aggregate with conspecifics (Parrish and Edelstein-Keshet, 1999). These aspects have not been studied among earthworms, yet both kind of density dispersal may potentially occur among them. In a final experiment, we focused on the effects of the pre-use of the habitat by conspecifics. Among most organisms, former occupation of a habitat is generally considered to have negative consequences on an actual population because previous inhabitants may have consumed a significant part of the resources and may increase the new comers intra-specific competition (Charnov, 1976). However, as earthworms are ecosystem engineers (Jones et al., 1994), they may also modify the habitat in a way that will benefit new arrivals.

2. Materials and methods

Four separate experiments were carried out, each of them addressing a specific question related to dispersal mechanisms. Experimental units consisted of rectangular areas; 1 m long, 0.18 m wide and 0.2 m high. The size of the mesocosms was estimated from data available (Edwards, 1998) and from preliminary experiments, so that they exceeded the estimated colonization rate in natural and artificial conditions for all species considered. Thus the mesocosms were large enough to give sufficient space for individuals, but also small enough, to make sure earthworms could cross the adverse section (see explanations below).

The experimental units were divided in three sections of identical dimensions (Fig. 1a): (1) An “inoculation section”, where earthworms were systematically introduced. It was filled with “suitable” or “unsuitable” soil, depending on the experiment (see details below in specific sections); (2) An “adverse section” filled with the “unsuitable” soil; (3) A “target section” filled with the “suitable” soil. This disposition is classical in studies of dispersal (e.g. Boudjemadi et al., 1999). The adverse section is fundamental as it allows distinguishing dispersal behaviour (patch departure) from diffusion behaviour (random movements, Nathan et al., 2008). Indeed, the adverse section prevents earthworms reaching the target section by simple random movements. In the absence of this section, earthworms would diffuse in the mesocosms from the release section until finding the best location. This would constitute a case of diffusion and habitat selection, but not dispersal sensu stricto. In the presence of the adverse section, reaching the target section requires some kind of decision to leave the inoculation section and to cross an inhospitable one. Hence in this case the response depends on the dispersal behaviour of the earthworms, not

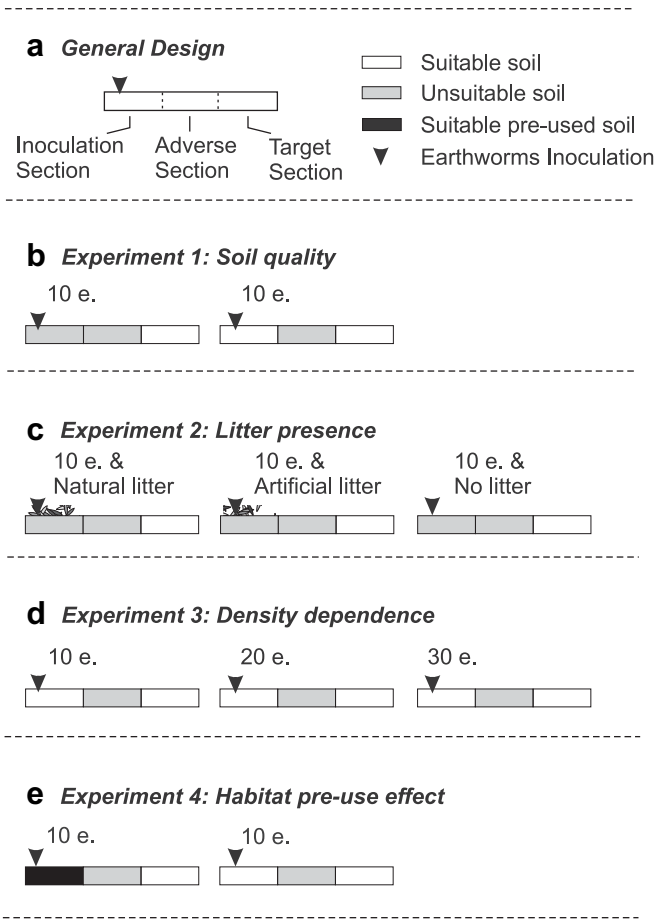

Fig. 1. Schematic representation of the experimental designs. The soil disposition in the mesocosms is symbolised by rectangles whose colours represent the nature of the soil substrate. The arrows indicate the section where earthworms (e.) were inoculated at the beginning of the experiments.

on the outcome of random movements. In consequence it is mandatory to separate the inoculation section from the target one by an adverse section. Inoculating the individuals in the middle of the mesocosms, without an adverse section, would give them equal access to the different soil types, and would constitute a case of pure habitat selection, with no dispersal process. In summary, the disposition with a release section and a target one separated by an adverse section is well adapted to properly test dispersal behaviour and to avoid confusion with diffusion and habitat selection.

The top of each experimental unit was covered by a nylon mesh for oxygenation and humidity conservation. Experiments were carried out in a glasshouse at the IRD Bondy centre, in France. Temperature was 18 °C during daylight and 15 °C during night, with 12 h of light per day and humidity was kept constant (soil humidity : 25% of dry weight). All experiments lasted one week and each treatment was replicated 6 times. We choose this period for two types of reasons. First, it appears to be relevant together with the size of our mesocosms in the light of the movement rates documented in the literature for earthworms (Mazaud and Bouché, 1980; Marinissen and Vandenbosch, 1992; Stein et al., 1992; Edwards and Bohlen, 1996). Second, we performed preliminary experiments that showed that earthworms are capable of crossing the mesocosms in one week.

2.1. Earthworm species

Earthworms are usually classified in three ecological categories: endogeic (that live and feed in the soil), anecic (that live in the soil but feed on surface litter) and epigeic (that live in and feed on surface litter) (Bouché, 1972). Due to their different ecologies, they face different constraints and might have evolved different dispersal strategies. We can expect epigeic species to be less sensitive to soil characteristics but more sensitive to the litter quality than endogeics. For this reason we used species of contrasted ecological categories: one endogeic: *Aporrectodea icterica* Savigny (1826) and one epigeic: *Dendrobaena veneta* Rosa (1886). The species names used herein conformed to the Fauna Europea web site (http://www.faunaeur.org/index.php).

A. icterica individuals were collected from the forest of Fontainebleau (48°24′N, 2°44′E). *D. veneta* individuals were purchased from a fishery shop. Earthworms were bred in the "suitable" soil at low density, at temperatures varying from 18 °C during the days to 15 °C at night. Earthworm individuals were used only once and replicates of each experiment were all performed simultaneously. All individuals were sub-adult at the time of the experiments.

2.2. Experiments

1) The influence of soil quality on endogeic dispersal was tested by comparing *A. icterica* displacements when 10 individuals were inoculated into "suitable" versus "unsuitable" soil (Fig. 1b).
2) The impact of litter cover, as shelter or as food resource, on epigeic dispersal was tested by comparing *D. veneta* movements after inoculating 10 individuals into three contrasted conditions: a) in "unsuitable" bare soil, b) in the same soil covered with *Tilia cordata* Miller (1768) leaf litter, and c) in the same soil covered with an artificial plastic litter (Fig. 1c).
3) Density dependence of endogeic dispersal was tested by introducing *A. icterica* at three different densities: 10, 20 or 30 individuals (respectively 166, 333 or 550 individuals m^{-2}) into the "suitable" soil (Fig. 1d). These density levels were representative of the natural levels observed for this species in the field (J. Mathieu, unpublished data)
4) The last experiment assessed the effect of former soil pre-use by conspecifics on endogeic dispersal rate. Ten specimens of *A. icterica* were inoculated (1) into the "suitable" soil that was previously processed by conspecific specimens, (i.e. the pre-used soil) or (2) into the same "suitable" soil but without pre-use (referred below as the "pristine soil") (Fig. 1e). The inoculation sections were prepared prior to the experiment: both soils were sieved one month before the experiment, and stored in boxes of the size of the inoculation sections. In the pre-used treatment, we inoculated 40 individuals of *A. icterica*. After one month they were removed by gently warming up the bottom of the boxes in a bain-marie. The soil for the pristine treatment was prepared in the same way but without earthworms.

2.3. Soils and litter

Two types of soil were used in the experiments to create habitats of different quality:

1) An "unsuitable" soil that was strongly avoided by earthworms during a previous preference test (Mathieu, unpublished data). This soil (Table 1) was sampled in a forest stand (48°24′N, 2°44′E, WGS84) dominated by *Quercus* sp. and *Carpinus betulus* and that contained very few earthworms.
2) A "suitable" soil that was largely preferred to the "unsuitable" soil in former preference tests. This soil (Table 1) was sampled in the park of the IRD Bondy centre (48°54′E, 2°29′N, WGS84), and contained more earthworms than the unsuitable soil.

Soils were sieved at 2 mm and re- humidified at respective field capacity by capillarity. They were adjacent in the three parts of the experimental units with no separation between them in order to allow earthworms to move freely from one section to the other. Removable partitions were used to avoid mixing of the section during their filling.

We also used two types of litter in the second experiment:

1) Leaves of *T. cordata* collected in the IRD Bondy park, at various decaying stages, up to one year old. This species was chosen because it is highly palatable to earthworms due do to its high Ca content (Reich et al., 2005).
2) A non-edible artificial litter to mimic the physical protection of natural litter but that could not be eaten. Artificial leaves were cut from thin (thickness: 5×10^{-4} m) plastic sheet and reproduced the shape of leaves at different decaying stages.

2.4. Statistical analyses

We defined the number of dispersing individuals as the number of individuals found in the target section at the end of the

Table 1
Selected properties of the soils used in the experiments. Unsuitable Soil = Soil avoided by the earthworms, Suitable Soil = Soil preferred by the earthworms.

Soil properties	Unsuitable soil	Suitable soil	Unit
Clay	4.7	15.7	%
Silt	18.5	13.4	%
Sand	76.8	70.9	%
Organic C	8.5	28.1	g kg^{-1}
Total *N*	0.33	2.61	g kg^{-1}
C:N	25.8	10.8	
Organic Matter	14.6	48.6	g kg^{-1}
pH	3.8	7.5	
CEC (Metson)	2.9	11.7	cmol kg^{-1}

experimental units. We analysed the link between the proportion of dispersers and the treatment with General Linear Models with Binomial response. All analyses were performed with R (R Development Core Team, 2007).

3. Results and discussion

3.1. Experiment 1&2: soil suitability and litter cover effects on dispersal

In the first experiment, 90% of the earthworms dispersed when inoculated into the unsuitable soil, whereas only 20% dispersed when inoculated into the suitable soil (Fig. 2). This striking difference shows that dispersal of *A. icterica* can be triggered by soil properties. In the second experiment, *D. veneta* responded dramatically to the presence of litter. When inoculated into bare ground, more than 80% of the individuals dispersed whereas only 26% dispersed when the inoculated section was covered by natural litter (Fig. 3). This significant difference shows that the presence of litter strongly influences the dispersal behaviour of this epigeic earthworm. Interestingly, less dispersal (34%) occurred in the presence of artificial litter than in bare ground (80%). The fact that both natural and artificial litter reduced dispersal suggests that the role of the litter as a shelter was more determinant than its role as a trophic resource.

A large body of observations already indicated that earthworms prefer habitats of high quality (in terms of food and environmental conditions) and that habitat quality actually affects earthworm fitness (Lowe and Butt, 2005). They also indicate that earthworms are able to select their habitat, and that they have food preferences (Westernarcher and Graff, 1987; Sanchez et al., 1997).

Our experiments highlight some kind of behavioural control in earthworm dispersal determinisms. Our results show that earthworm can disperse even if they are surrounded by an adverse environment, while there is no evidence of immediate benefits to disperse. This suggests that in the field, earthworms may avoid unsuitable environments and move until reaching a better habitat. Therefore earthworms should be more abundant in high quality habitats i.e. with high organic matter content, sufficient litter cover, or suitable soil properties. This prediction is generally verified for epigeic species (Westernarcher and Graff, 1987; Cannavacciuolo et al., 1998), but not always for endogeics (Valckx et al., 2009). Indeed, at small scales, typically plot scale of a few ha, the distribution of endogeic earthworms often display aggregative patterns forming patches with high densities (Margerie et al., 2001; Rossi, 2003), sometimes stable over periods of 2–3 years (Decaëns and Rossi, 2001; Jimenez et al., 2006). These patches are not consistently related to organic matter distribution (Rossi et al., 1997),

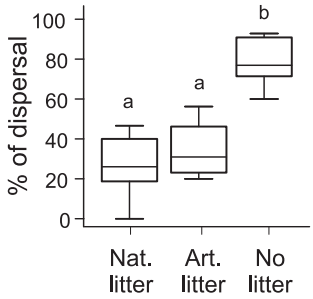

Fig. 3. Box plots comparisons of *D. veneta* dispersal rate in response to litter cover. Nat. = Natural litter; Art. = Artificial litter; different letters indicate significant differences at $p = 0.05$ (General Linear Models with Binomial response).

which suggests that soil properties other than organic matter may influence their location, and also that mechanisms other than habitat selection and dispersal from low quality habitats may be involved in the distribution of earthworms at the plot scale.

3.2. Experiment 3: density dependent dispersal

Dispersal rate of *A. icterica* increased with the density inoculated into the release section, with values significantly different between the lowest and highest density levels. Dispersal rate was 40% in treatments with 10 individuals inoculated, 45% with 20 individuals, and 69% in treatments with 30 individuals (Fig. 4). This endogeic species therefore seems to present a strong positive density dependent dispersal, a mechanism that has never been described in earthworm population studies, although it has been proposed to explain the punctual massive migration observed for some species (Reddy, 1980).

Positive density dependence in dispersal behaviour is supposed to be widespread in animals (see for instance Matthysen, 2005), and available examples include some soil organisms (see for instance Bengtsson et al., 1994). The most widely acknowledged hypothesis is that crowding increases intra-specific competition due to resource depletion, and that better fitness should be attained by dispersing from high-density sites (Murray, 1967; Waser, 1985). It was also noticed that positive density dispersal can avoid attracting predators in patches of high prey density (Wittenburger and Hunt, 1985), a phenomenon reported on earthworms (Macdonald, 1983). However, this behaviour can present serious evolutionary drawbacks, which may outweigh the benefits of positive density dispersal, especially among earthworms. In particular, emigration may induce local Allee effects (Stephens and Sutherland, 1999) which can seriously impede the growth and survival of populations. Many species of insects, birds

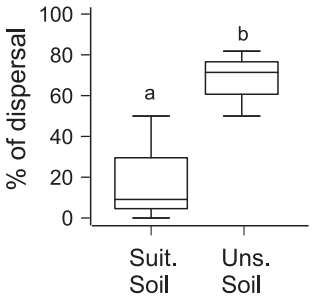

Fig. 2. Boxplot comparisons of *A. icterica* dispersal rate in response to soil properties. Suit. = Suitable soil; Uns. = Unsuitable soil; different letters indicate significant differences at $p = 0.05$ (General Linear Models with Binomial response).

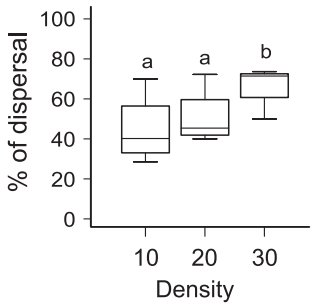

Fig. 4. Boxplot comparisons of *A. icterica* dispersal rate in response to earthworm density (number of individuals) inoculated into the soil. Different letters indicate significant differences at $p = 0.05$ (General Linear Models with Binomial response).

and mammals present negative density dependence, which often results from conspecific attraction (Danielson and Gaines, 1987; Stamps, 1991). The aggregation behaviour was already reported among the earthworm species *Lumbricus terrestris* (Linné, 1758), but the effect could not be distinguished from habitat selection (Butt et al., 2003). Indeed, in this experiment aggregation might come from a natural tendency to aggregate without a forcing by the heterogeneity of the environment, i.e. an aggregation behaviour, but also from individuals ending up in the patch of high quality after selecting the best habitat available (habitat selection).

3.3. Experiment 4: soil pre-use effect on dispersal

Soil pre-use strongly reduced the dispersal rate of earthworms: in pristine soil dispersal rate was 30%, whereas in pre-used soil no individuals dispersed (Fig. 5). This result shows that earthworm activities can have persistent effects which can be detected by new immigrants. This result may be explained by different mechanisms. First, the former inhabitants may have increased the quality of the habitat through soil engineering, i.e. the burrowing of galleries, which is a highly energy consuming activity. New comers would thus prefer soil with existing galleries, which would represent a readily suitable habitat colonisable with minimal burrowing cost. This hypothesis is supported by a recent work on earthworms behaviour (Felten and Emmerling, 2009). Second, former inhabitants may have enhanced trophic resource quality by activating decomposition processes through soil ingestion and mucus deposition, a mechanism previously coined “external rumen” (Lavelle, 1986). This kind of priming effect increases the availability of nutrients and carbon for the next consumers. Lastly, earthworms may have released attracting molecules in the soil. For instance, some anecic earthworms leave mucus on the ground which they use to locate their burrows (Nuutinen and Butt, 1997). This mucus may behave as a signal molecule which attracts conspecifics, a point which as never been tested, including on endogeics species, on which we worked.

This positive effect of soil pre-use can be considered as an original form of philopatry (i.e. the tendency to return to a specific environment or location) where the environment is the by-product of former individual activity. Such complex feedbacks between habitat quality, engineering activity, and dispersal have already been mentioned in theoretical works, but have rarely been demonstrated experimentally (see Cuddington and Hastings, 2004; Klironomos, 2002). Theoretically they can lead to the formation of patches of individuals through self-organization, without the forcing of any pre-existing heterogeneity in soil properties or interspecific interactions (Barot et al., 2007).

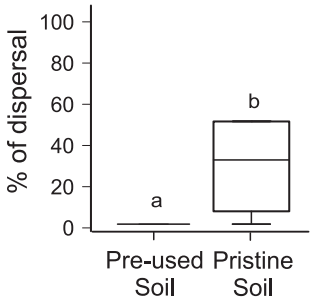

Fig. 5. Box plots comparisons of *A. icterica* dispersal rate in response to the former use of the soil substrate by conspecific individuals. Different letters indicate significant differences at $p = 0.05$ (General Linear Models with Binomial response).

3.4. Dispersal behaviour and earthworm spatial distribution

Dispersal patterns have profound effects on the distribution of species and community assemblage (Holyolak et al., 2005; Decaëns et al., 2008). Long Distance Dispersal (LDD) is a condition for the maintenance of metapopulations and metacommunites (Holyolak et al., 2005), and determines the capacity of species to colonize free habitats. At this scale, movements are likely dominated by passive dispersal mediated by external factors such as animals, wind, runoff and human activities. For instance earthworm cocoons may be transported in the fur of animals, in the soil of potted plants, or by being stuck in tractor wheels (Marinissen and Vandenbosch, 1992).

However passive dispersal alone is probably not sufficient to explain earthworm distributional patterns. Also active dispersal potentially plays an important role even at large scale. For instance the accumulation of many small stepping stone displacements can lead to large migration patterns or invasions (Nentwig, 2007). Many invasive species that were first introduced artificially in remote areas spread themselves by active dispersal (Lockwood et al., 2006). Some rare observations of introduction in previously earthworm-free habitats, such as polders, showed that earthworms are capable of colonising new areas at distances ranging from 4 (*L. terrestris*) to 14 m year^{-1} (*Lumbricus rubellus* Hoffmeister (1843)) (Mazaud and Bouché, 1980; Marinissen and Vandenbosch, 1992; Stein et al., 1992; Edwards and Bohlen, 1996). According to our experiments, *A. icterica* and *D. veneta* can travel distances of 0.5–0.9 m per week in mesosoms (26–47 m. year^{-1}) under conditions that trigger dispersal. Massive and spectacular migrations of earthworms are acknowledged to occur episodically (Reddy, 1980), but they probably don't occur very often.

At small scales, earthworms are known to form patches of high densities separated by areas of low densities (Rossi et al., 1997). The formation of these patches is a complex phenomenon resulting from local demographic processes associated with migration – emigration (both aspects of dispersal), interspecific interactions (competition versus ecological complementarity) and feedbacks between soil quality and earthworm engineering activity (Barot et al., 2007; Decaëns and Rossi, 2001; Rossi et al., 1997; Jimenez et al., 2006; Decaëns et al., 2009). In theory, their formation may arise from different processes, the most evident being an aggregation behaviour. However we observed rather positive density dispersal, meaning that earthworms avoid high densities. This implies that earthworms are relatively mobile at small scale, a condition under which patch formation was not expected in previous models, unless there was a strong influence of soil properties on demographic parameters (Barot et al., 2007). Under these circumstances patch formation appears as a subtle quantitative output of the balance between local demography and dispersal behaviour. Therefore in order to explain the formation of patches of earthworms, it is necessary to evaluate their movement range, their dispersal kernel as well as the variability of their demographic parameters in the field. In addition to these intra-specific mechanisms, interspecific interactions should also be considered. Competition, ecological complementarity and facilitation interactions may be in part responsible for spatial patterns observed in earthworm assemblages. For instance, interspecific competition has been described as a potential driving factor for the formation of patches dominated by specific species assemblages (Holyolak et al., 2005; Jimenez and Rossi, 2006; Decaëns et al., 2009), which is supported by experimental results that demonstrated antagonistic interactions between species pairs (Butt, 1998). Conversely, mutualistic, or at least reciprocal beneficial relationships can also lead to patch formation (Hoopes et al., 2005). For instance it was thought to explain the distribution of two African species in separate patches, where each species was

relying on the activity of the other to access soil organic matter (Blanchart et al., 1997).

From our result we can elaborate a first behavioural scenario of earthworm foraging: when they are either in a crowded or a low resource patch, they disperse until finding a suitable place, preferably formerly inhabited by conspecifics. After some time density increases in the suitable places, leading potentially to patch formation, and after some more time, density starts decreasing because of positive density dispersal.

3.5. Concluding remarks and perspectives

Our work clearly shows that earthworms are reactive to the quality of their environment, and that they can easily disperse from unsuitable conditions. The mechanisms we highlight bring significant information but they are not sufficient to explain patch formation in field conditions, and should thus be considered in tandem with field demographic studies.

Further work is required to understand how dispersal influences population and community dynamics. In particular, it is necessary to investigate if all species behave similarly in response to the same environmental conditions. Indeed, species, or even earthworm ecological categories, may present specific dispersal behaviours. The second point we need to focus on is the role of life stage. Indeed hatchlings, juveniles and adults may have very different behaviours and hence different dispersal options, as suggested by previous studies (Cannavacciuolo et al., 1998; Valckx et al., 2009). Lastly, the effects of interspecific interactions on dispersal behaviour should be considered. Species distribution in the field likely depends on this aspect. In order to model earthworm dispersal in a realistic way, we also needs to evaluate the mathematical shape of the dispersal kernel (dispersal curve) – the frequency distribution of the distances travelled by all individuals in a population –, which is a central feature of classical dispersal models (Kot et al., 1996; Neubert and Caswell, 2000). This could be done by taking advantage of recent techniques, such as earthworm tagging (Fujiwara et al., 2006; Butt and Lowe, 2007) and genetic approaches (Manel et al., 2003), allowing the study of individual movements at both small and large scales (Nathan, 2003).

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Annexe 9

Caro G., Hartmann C., Decaëns T., Barot S., Mora P., Mathieu J. Impact of soil engineering by two contrasting species of earthworms on their dispersal rates, Applied Soil Ecology, 2014, 84:223-230

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Impact of soil engineering by two contrasting species of earthworms on their dispersal rates

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ABSTRACT

By burrowing galleries and producing casts, earthworms are constantly changing the structure and properties of the soils in which they are living. These changes modify the costs and benefits for earthworms to stay in the environment they modify. In this paper, we measured experimentally how dispersal behaviour of endogeic and anecic earthworms responds to the cumulative changes they made in soil characteristics. The influence of earthworm activities on dispersal was studied in standardised mesocosms by comparing the influence of soils modified or not modified by earthworm activities on earthworm dispersal rates.

The cumulative use of the soil by the earthworms strongly modified soil physical properties. The height of the soil decreased over time and the amount of aggregates smaller than 2 mm decreased in contrast to aggregates larger than 5 mm that increased. We found that: (i) earthworm activities significantly modified soil physical properties (such as bulk density, soil strength and soil aggregation) and decreased significantly the dispersal rates of the endogeic species, whatever the species that modified the soil; (ii) the decreasing in the dispersal proportion of the endogeic species suggests that the cost of engineering activities may be higher than the one of dispersal; (iii) the dispersal of the anecic species appeared to be not influenced by its own activities (intra-specific influences) or by the activities of the endogeic species (inter-specific influences). Overall these results suggest that the endogeic species is involved in a process of niche construction, which evolved jointly with its dispersal strategy.

1. Introduction

Active dispersal of animals is a central ecological process that allows habitat colonization and the exploitation of resources that vary in time and space (Ronce, 2007). It is therefore regarded as a key process that determines species distribution from the local to the biogeographical scale (Hengeveld and Hemerik, 2002; Eijsackers, 2010, 2011; Mathieu and Davies, 2014). As a consequence, the study of dispersal has become a major field of research in ecology (Nathan, 2003). As of the direct relationship between dispersal behaviour and fitness, a wealth of literature has focused on the evolution and consequences of dispersal capacities. A central issue is the need to determine the conditions that induce dispersal (Matthysen, 2012). Dispersal behaviour involves the departure from a breeding site, moving to a new place, and settlement, and can occur at any life stage, at any spatial scales above the individual range and within more or less heterogeneous landscapes (Clobert et al., 2009). A recurrent finding of evolutionary models is that dispersal rates are mainly determined by a balance between dispersal costs and benefits (Bowler and Benton, 2005) that depend on environmental factors (e.g. habitat quality, habitat fragmentation, patch size, density, predation) (Bonte et al., 2012). We can therefore hypothesise that organisms that modify their physical and chemical environment through their activities, the so-called ecosystem engineers (Jones et al., 1994), modify the costs and benefits of their own dispersal. Through the modifications they impose to their environment they could therefore modify their own dispersal rates.

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If engineers improve the quality of their environment, we can expect that they should benefit from reducing their dispersal rates from patches they have engineered (i.e. they stay longer in engineered habitat). This would constitute a positive feedback (Mathieu et al., 2010). Conversely, if engineers decrease the quality of their environment they should benefit from increasing their dispersal rates from these patches (Caro et al., 2013a). This would constitute a negative feedback. Therefore, documenting the impact of habitat changes imposed by engineers on their own dispersal rates should help showing whether there is a negative or positive feedback between the engineer and its habitat, and it should give simultaneously key information on the dynamics of both engineer population and its habitat.

Feedback between organisms and their environment has been studied in plants (Kulmatiski et al., 2008), where they have been shown to be influential for plant demography and spatial distribution, species successions and coexistence patterns (Barot and Gignoux, 2004). Some models also confirm that feedback between ecosystem engineers and their environment may affect their demography and distribution and that this feedback is affected by the mobility of the engineers (Barot et al., 2007; Raynaud et al., 2013). Here we tested if earthworm active dispersal may be influenced by earthworm-mediated engineering activities. Such a mechanism has been, to our knowledge, poorly studied and is likely to affect the strength of the feedback between the engineer and its environment and to influence its spatial distribution.

Earthworms are considered as key ecosystem engineers in the soil system (Lavelle et al., 2006). It has been shown that dispersal rates of *Aporrectodea* icterica can be reduced by the activities of conspecifics, whereas its dispersal rates increase with conspecific densities, as other earthworm species (Mathieu et al., 2010; Caro et al., 2013a). These apparently contradictory results suggest the existence of complex feedbacks between soil quality, engineering activities, and dispersal. In the field, communities of earthworms can indirectly interact through modifications of their common habitat, i.e. the soil. It is therefore necessary to evaluate the influence of interspecific interactions through earthworm activities on their dispersal rates. Earthworms often have patchy distributions (Richard et al., 2012). Such distributions are characterized by high earthworm densities in some patches, which consequently locally increases intensity of soil use by earthworms. According to our rationale and previous observations (Mathieu et al., 2010; Caro et al., 2013a), dispersal rates of earthworms should be impacted by the high density in these patches. Testing for such an effect and determining its influences is necessary to understand and predict earthworm dynamics and their spatial distribution.

To tackle the issue of the impact of habitat use by soil earthworms on their own dispersal, an experiment was established to determine how earthworm intra- and inter-specific activities affect soil properties and in turn dispersal rates. We characterized the soil physical, chemical and biological changes induced by the activities of two earthworm species, *Aporrectodea giardi* and *A. icterica* (Bouché, 1972, 1977). In the rest of the paper, we refer to earthworm activities as engineering activities. Further, we investigate how these changes influence the dispersal behaviour of each species.

2. Materials and methods

2.1. Earthworms

To observe the dispersal behaviour of an earthworm species in response to (i) its own activity or (ii) to the activity of another species, we used two species that co-exist in natural conditions: *A. giardi* (Ribaucourt 1901) and *A. icterica* (Savigny 1826). These two species differ by their size and feeding behaviour. *A. giardi* is the largest one with a length ranging 130–170 mm and a weight of 3.3 ± 0.9 g; it is an anecic species, i.e. feeding on surface litter. *A. icterica* is approximately to folds smaller with 70–90 mm length and three folds lighter with a weight of 1.2 ± 0.25 g; moreover it is an endogeic species feeding on organo-mineral soil. Adults of both species were sampled in grasslands in the centre of France (48.6167 N, 1.6833 E). They were reared in a pasture soil maintained at 15 °C during the day and 10 °C at night, we used horse dung to feed them. For the experiment, each individual was used only once.

2.2. Soils

We used two different soil types (Mathieu et al., 2010; Caro et al., 2013a): (1) a sandy soil collected in the forest of Fontainebleau (48.413287 N, 2.748245 E) that represented an "unsuitable" habitat for earthworms as it contained no earthworm in field conditions in relation with adverse physical and chemical characteristics (pH 3.8, organic carbon content = 0.85% and C:N ratio = 25.8); (2) a loamy soil collected in a grassland (48.91431 N, 2.484806 E) that represented a "suitable" habitat as it contained both species in natural conditions in relation with favourable soil characteristics (pH 7.5, organic carbon content = 3.91% and C:N ratio = 17). More information on these soils can be found in (Mathieu et al., 2010; Caro et al., 2013a). We collected 800 kg of the unsuitable and 1600 kg of the suitable soils both were air-dried for 4 days. The total 2.4 t of soil was sieved at 2 mm and this fine soil was rewetted to 0.25 g water g^{-1} dry soil.

2.3. Experimental design

The experiment had two main steps: firstly the fine soil was first engineered by one of the two species; secondly we observed the effect of the engineered soil on the dispersal rates of the both species.

2.3.1. Soil engineering by the earthworms (step 1.1)

Only the suitable soil was used. It was put in 5 L containers (33 cm long, 15 cm wide and 10 cm high) with an initial bulk density of 1 g/cm^3; horse dung was uniformly added at the surface (150 ± 1 g in each container). A total of 180 containers were prepared (Fig. 1, step 1):

- $N = 20$ containers used at T0 (10 for each earthworm species);
- 160 containers at the other durations; i.e. 40 containers used at each of the 4 durations (1, 2, 4 and 6 weeks): $N = 10$ being inoculated with *A. giardi*, $N = 10$ inoculated with *A. icterica* and $N = 20$ without worms used as controls.

The layout of the 180 containers was spatially randomized. In the inoculated containers, we introduced 30 adult individuals, i.e. 6 individuals L^{-1}. This earthworm densities used may be high in comparison to field conditions, however such densities where required for the soil to be significantly engineered within a short time. In the field, earthworms may engineer the soil for months but, for practical reasons, such duration was not possible for the pre-experiment.

2.3.2. Removing earthworms (step 1.2)

At the end of the engineering period, we weighted the mass of the remaining dung. Then, earthworms were removed without disturbing the soil physical structure and without altering earthworm health: the plastic containers were dived in a hot water bath (60 °C). While the soil temperature was slowly increasing, the earthworms came at the surface and were caught manually and weighed individually. The controls containers were similarly dived in the hot

water bath. After all earthworms were caught, soil height in the container was measured to calculate the new soil bulk density and we measured the mechanical resistance to penetration (see below). Finally, the soil material was translocated to the mesocosms without disturbing its physical structure.

2.3.3. Setting up dispersal mesocoms (step 2.1)

The mesocosms consisted in a dispersal corridor (100 cm long, 15 cm wide and 10 cm high), divided in three equal sections (Fig. 1, step 2): (1) the "inoculation section" filled with the soil engineered by the earthworms for various durations; (2) the "crossing section" composed of unsuitable soil; (3) the "arrival section" composed of suitable soil sieved at 2 mm. The crossing section was determinant because it represented a physical barrier that generated dispersal costs, and thus allowed only active dispersal and avoided diffusion (i.e. random movements with possible returns to the starting point) (Caro et al., 2012).

We added 10 earthworms in the inoculation section containing the engineered or the control soil (Fig. 1, step 2). We made four combinations to test intra-and inter-specific influences:

- intra specific influences: *A. icterica* individuals in the soil engineered by *A. icterica* (II) and *A. giardi* in the soil engineered by *A. giardi* (GG).
- inter specific influences: *A. giardi* in the soil engineered by *A. icterica* (GI) and *A. icterica* in the soil engineered by *A. giardi* (IG) (Fig. 1).

For each treatment, we made $N = 5$ replicates and $N = 5$ controls.

2.3.4. Measurement of the dispersal rate (step 2.2)

After seven days, each of the three sections was physically isolated from the others, and in each section the earthworms were counted and weighed individually and the dispersal rate (% disp.) was calculated as the proportion of individuals that reached the arrival section. We measured the physical and chemical characteristics of the soil from the inoculation section.

2.4. Measurements of soil physical and chemical properties

Bulk density was calculated as the weight of the soil in the container divided by its volume. Soil strength was quantified with a penetrometer consisting of a 3 mm rod mounted on a mobile base and connected to a pressure sensor. The rod was pushed into the soil with a constant velocity (0.067 mm s^{-1}); the penetration resistance was measured at regular intervals (0.1 mm) for the entire soil height. In each container, an average resistance profile was calculated by transect of 5 replicates along each container. The slope of the linear regression between penetration resistance and depth was considered as the soil strength (*Rc*). We measured the aggregate size distribution by passing an aliquot of 1 kg of air-dried soil through a set of sieves (10, 5 and 2 mm mesh sizes). The soil remaining on each sieve was weighed to obtain the proportions of aggregates >10 mm, 10–5 mm, 5–2 mm and <2 mm. Soil water content was calculated by estimating the mass loss observed after drying a 100 g aliquot of soil for 48 h at 105 °C.

pH was measured on a suspension of 10 g of air-dried soil in 50 ml water (ISO 10390:2005). C and N contents were measured by dry combustion (ISO 10694:1995; ISO 13878:1998), P was quantified by the Olsen method (ISO 11263:1994).

2.5. Statistical analysis

We used ANOVA to analyse the effects of the earthworms on soil properties for most characteristics. For soil strength, an increase can result from increased bulk density or a change in soil structure due to engineering activity. Thus, we performed a Pearson correlation between the *Rc* values and soil height to determine whether soil strength was the result of earthworm

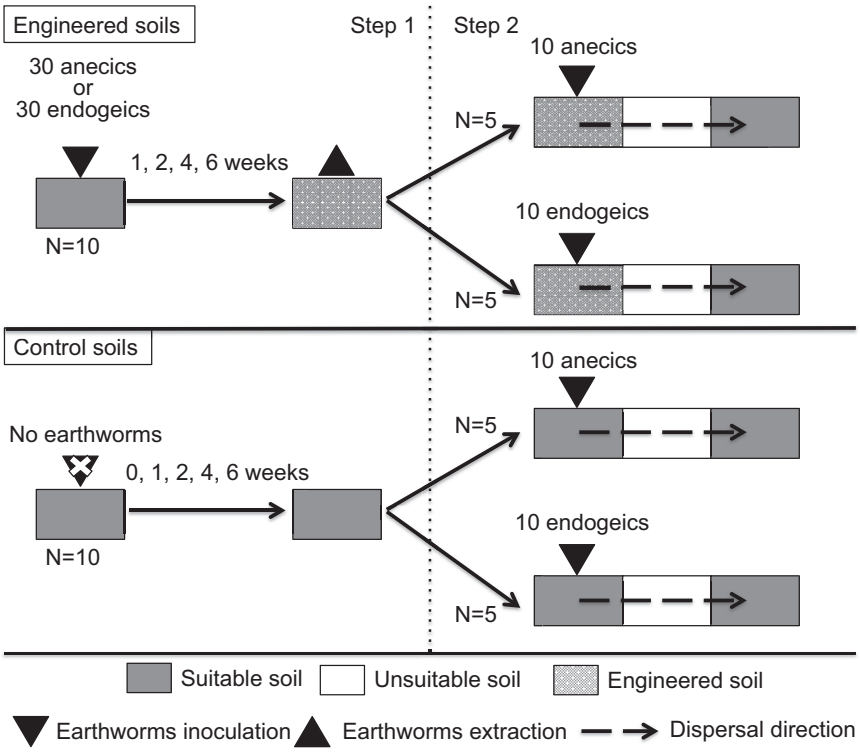

Fig. 1. Experimental design of the study. Step 1 corresponds to the engineering of the soil by earthworms; step 2 corresponds to the dispersal experiment per se.

activities or of a natural collapse with time. The absence of correlation indicated that soil strength resulted from earthworm activities.

To compare the dispersal rates across the engineering periods, we used a General Linear Model (GLM) with a binomial family. To determine which soil parameters significantly influenced earthworm dispersal rates, we performed a multiple linear regression between dispersal rates of both species and soil characteristics that were significantly affected by earthworm activity. With a stepwise procedure, the Akaike Information Criterion (AIC) was used to select the most relevant model (Burnham et al., 2011). Then we selected the variables that composed the model with the lowest AIC and analysed their influence on dispersal rates with non-linear regression, using the following equation:

$$D(X_i)_j = a_j \times \exp(-b_j \times X_i),$$

where:

- $D(X_i)_j$ represents the dispersal rate (%) of species j in response to the soil parameter X in the soil engineered by species i;
- a_j represents the maximal dispersal rate (%) of species j in an un-engineered habitat;
- b_j represents the influence of the soil parameter X engineered by species i on the dispersal rate of species j.

Note that i and j can represent the same species, so the approach allowed us to test both the intra- and inter-specific interactions mediated by earthworm activities. To quantify the influence of a soil parameter X on dispersal, we calculated the coefficient of determination between the dispersal rate and the soil parameter X. Then we assessed its significance by testing the differences between this coefficient of determination and a null model with random intercept only by using an ANOVA.

3. Results

3.1. Soil properties

The activities of both species significantly affected the soil physical properties in the same way. Between T0 and T6, bulk density increased significantly by 30.4% ± 14.7 and by 22.8% ± 6.6 in presence of *A. giardi* and *A. icterica,* respectively (Fig. 3a). This increase was significantly higher with *A. giardi* than *A. icterica* during the first two weeks of the experiment, while no difference was observed for longer time periods (Fig. 3a).

Both species significantly increased the soil resistance over time. Over 6 weeks (T6), soil height was reduced by 27% ± 12 and by 20% ± 7 with *A. giardi* and *A. icterica* respectively (Fig. 2). Between T0 and T2, *A. giardi* compacted the soil more intensively than *A. icterica* (Fig. 3b) but after T4, a higher compaction was observed with *A. icterica* (Fig. 3b). Consequently, the *Rc* value reached a maximum of 0.02 ± 5.10^{-3} with *A. giardi*, whereas it increased steadily during the experiment with *A. icterica* (Fig. 3b). In the controls (without earthworms) and *A. giardi* treatments, soil penetration resistance was correlated to soil height (p-value < 0.01) contrarily to *A. icterica* (p-value > 0.05).

The both earthworm species consumed the horse dung at the soil surface. *A. giardi* (the anecic species) has consumed 100% of the horse dung after 2 weeks (T2) whereas *A. icterica* (the endogeic species) has consumed 59% ± 6 after 6 weeks (T6). It is noteworthy that a significant loss of weight was measured for *A. giardi* (−23% ± 3) after 4 weeks (T4), whereas no variation was observed for *A. icterica*. However, no relation between food consumption and weight loss, or soil properties or dispersal rates was found (p-value > 0.05).

Both earthworm species significantly influenced the soil aggregate size distribution (Fig. 3c and d). The proportion of 5–10 mm aggregates increased significantly by 24% ± 3 and by 16% ± 3 with *A. giardi* and *A. icterica* respectively (Fig. 3c). The proportions of aggregates <2 mm decreased significantly by 30% ± 12 and by 19% ± 11 with *A. giardi* and *A. icterica* respectively (Fig. 3d). No difference was observed for the 2–5 mm and >10 mm aggregate size classes. The earthworms did not affect the chemical properties that we measured (p-value > 0.05).

3.2. Dispersal rates

Soil engineering significantly decreased the dispersal rates of *A. icterica* when it did not influence the dispersal rate of *A. giardi*

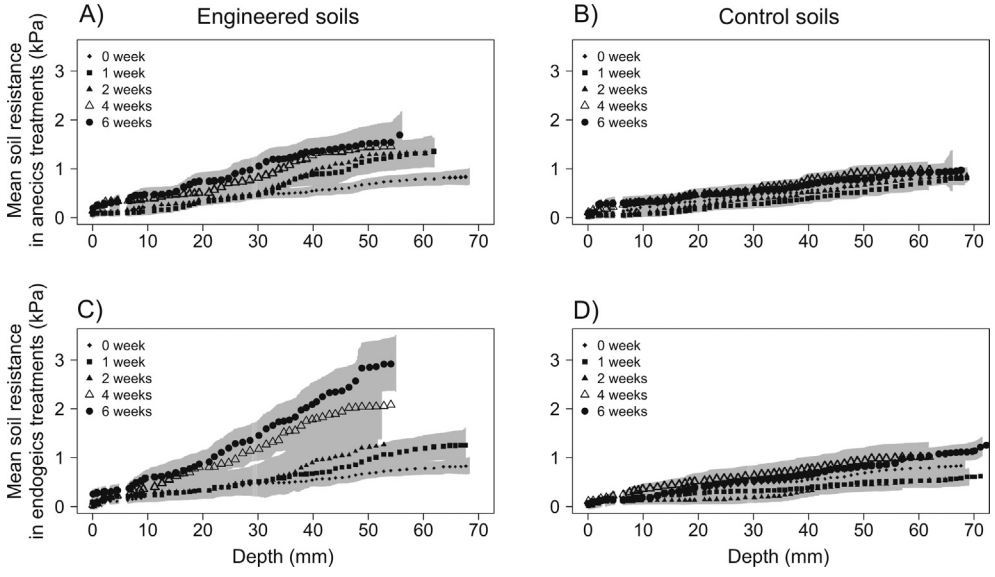

Fig. 2. Mean soil strength variation with soil height in the different treatments: (A) soil engineered by the anecic species (*Aporrectodea* giardi), (B) control for the anecic treatment, (C) soil engineered by the endogeic species (*Aporrectodea* icterica), (D) control for the endogeic treatment. Grey area = standard deviation.

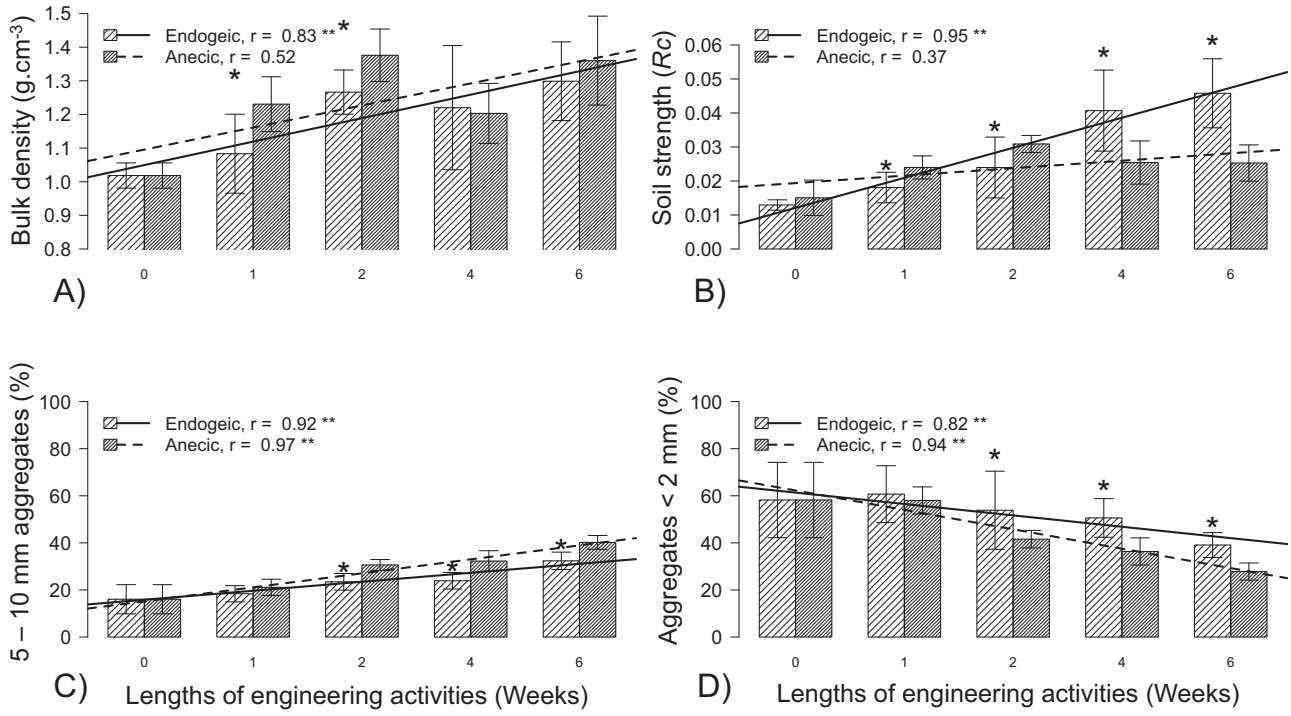

Fig. 3. Dynamics of (A) bulk density, (B) *Rc* value, (C) proportion of 5–10 mm aggregates and (D) proportion of aggregates smaller than 2 mm, as affected by earthworm activities (mean ± standard deviation, $N = 10$). Lines represent linear regression between soil properties and experimental duration; * indicates significant difference between the treatments with the anecic (*Aporrectodea giardi*) and endogeic species (*Aporrectodea icterica*), at p-value < 0.05; ** indicates significant r^2.

(Fig. 4). Using the AIC criterion, we found a relation between dispersal rate and (i) soil penetration resistance (*Rc* value) and (II) the proportion of 5–10 mm aggregates. Dispersal rate for *A. icterica* decreased when individuals were inoculated in engineered soil (Figs. 5 and 6). In the "II" and "IG" treatments, dispersal rates were more strongly correlated to the proportion of 5–10 mm aggregates than to *Rc* ($r^2 = -0.56$ and -0.42, respectively; Figs. 5 and 6). For *A. giardi's* dispersal rates, no relationship with soil physical properties was observed (Figs. 5 and 6) and no significant effect of chemical properties was observed.

4. Discussion

4.1. Earthworm activities influenced soil properties

Earthworms changed soil physical properties in a way that can be explained by burrowing and cast production (Lavelle et al., 2006; Capowiez et al., 2012). In the condition of the experiment, changes in soil structure solely due to physical processes, without earthworm activity, were insignificant (Fig. 3b and d). The earthworm activities impacted soil structure in a way that was

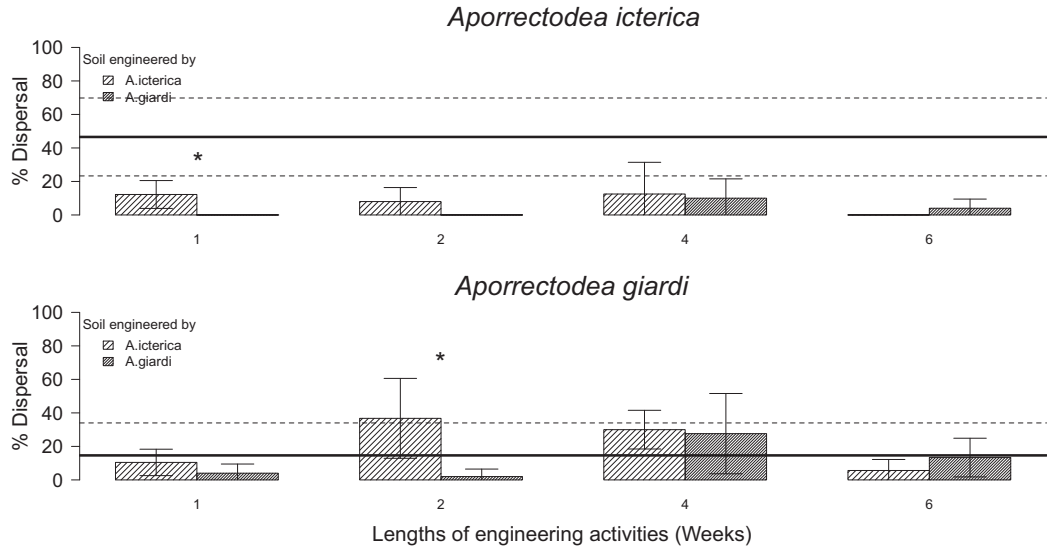

Fig. 4. Dispersal rates in response to the duration of the pre-experimental period of soil engineering by earthworms ($N = 5$). The horizontal solid line represent the mean dispersal rate of all controls and the horizontal dashed lines represent standard deviation; * indicates significant differences at p-value = 0.05 (GLM with binomial response) between the two juxtaposed barplots; lightly shaded barplots represent dispersal rate from a soil engineered by *Aporrectodea icterica* and heavily shaded ones represent dispersal rate from a soil engineered by *Aporrectodea* giardi.

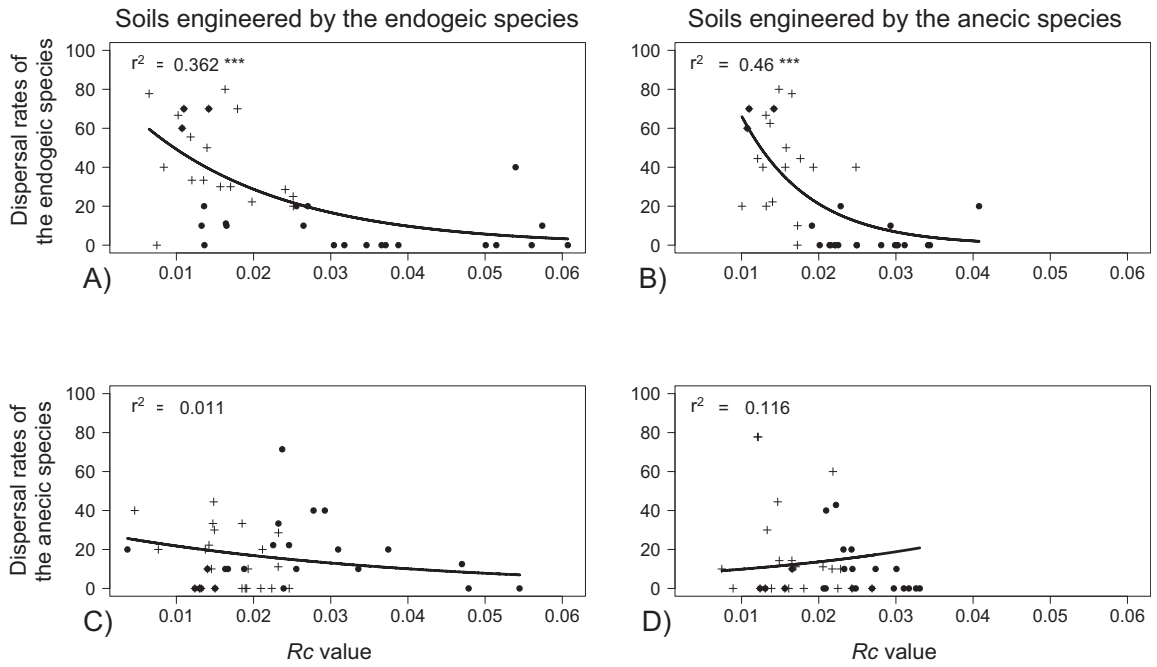

Fig. 5. Correlations between earthworm dispersal rates and *Rc* values. Points = engineered treatment; cross = controls. Solid line = non linear regression by fitting $D(X_i)_j = a_j \times \exp(-b_j \times X_j)$. *** = significant difference between the fitted model and a null model using an ANOVA.

qualitatively and quantitatively similar to what was previously observed in the field (Blanchart et al., 1999; Frelich et al., 2006). In the engineered soil, the structural changes could thus be attributed to earthworm activities only, i.e. gallery burrowing and cast production. Despite the ecological differences between the two species (burrowing and feeding behaviours), some similarities were observed in the structural changes due to their activities: (i) a decrease in the total pore volume; (ii) a global soil compaction and; (iii) an increase in the proportion of large aggregates associated to a decrease in the proportion of small aggregates. The large aggregates were most probably resulting from the association of the smaller ones in the wall of the galleries and in the casts. Despite these similarities, an important difference can be observed in the mechanical resistance: the increase in soil resistance induced by *A. icterica* was almost twice more important than the one induced by *A. giardi* (Fig. 3b).

The absence of effect on soil chemical properties may be due to the short duration of our experiment, as also observed for carbon and nitrogen contents for periods greater than several months by Pashanasi et al. (1996) and Edwards (2004). The dung consumption

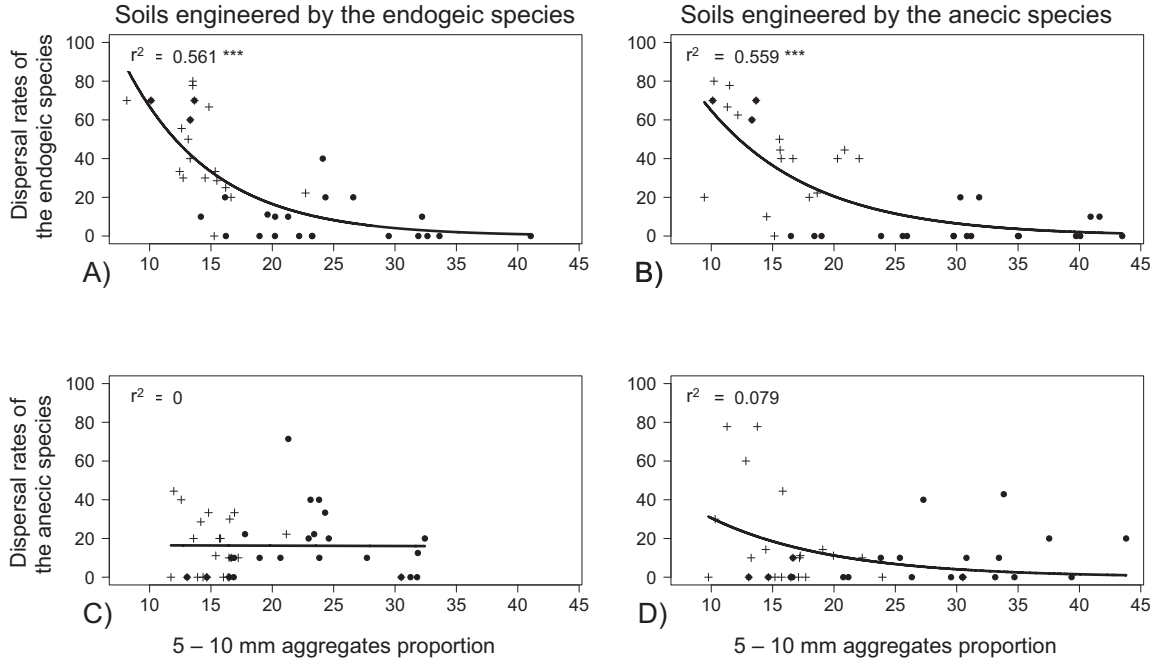

Fig. 6. Earthworm dispersal rates in response to the proportion of 5–10 mm aggregates. Points = engineered treatment; cross = controls. Solid line = non linear regression by fitting $D(X_i)_j = a_j \times \exp(-b_j \times X_j)$. *** = significant difference between the fitted model and a null model using an ANOVA.

resulted in the incorporation of dung in the soil. The absence of effect on soil properties and on dispersal rate suggests that in our case the incorporation of dung did not influence soil properties and earthworms dispersal. Nevertheless, it is possible that in the field, at least in some cases, earthworm impact on litter decomposition and on the incorporation of litter into the soil profile influences earthworm dispersal (Mathieu et al., 2010).

4.2. Earthworm dispersal rates changed along the use of the soil by earthworms

Dispersal rate of the endogeic species decreased significantly with the proportion of 5–10 mm aggregates and soil strength. With 10 individuals, dispersal occurs at a low rate from the inoculation section fulfilled with control soil (the not engineered one, Caro et al., 2013a), so that changes in soil properties could lead to changes in the dispersal rate. Such an earthworm density is thus particularly suitable to test the impact of soil engineering on dispersal, which is precisely our goal. We did not observe an increase in dispersal rate when soil had been strongly used. This suggests a significant feedback between the way this species physically engineers the soil and the drivers of its own dispersal rate: by modifying the soil, individuals inhibits the environmental stimuli generating dispersal movements. The absence of stimuli triggering dispersal should increase the density of soil engineers and further increases soil engineering. This engineering, i.e. changes in soil aggregation, might increase the habitat quality for earthworms (cues, casts or galleries presence). Indeed, the structures existing in engineered soil (for instance, galleries) might facilitate movement of earthworms and so reduce dispersal costs (Caro et al., 2012).

In the case of the anecic species, we found no response to soil engineering, irrespective of the two species that engineered the soil. We previously observed that the combination of both intra-specific and soil engineering effects influences significantly *A. giardi* dispersal across the time (Caro et al., 2013b). Here, no stimulating effect of soil engineering by a high density of conspecific earthworms on the *A. giardi* dispersal rates was observed. The comparison between the both studies suggested that only the combination of direct intra-specific interactions and soil engineering may affect the dispersal of *A. giardi*.

4.3. Niche construction mechanism in endogeic species?

A decrease in dispersal rate in response to habitat engineering may suggest an increase in habitat quality: the earthworms stay in the soil they have engineered only if they benefit from soil engineering. Our observations suggest the existence of such a feedback for the endogeic species (see also Mathieu et al., 2010). Dispersal rate is assumed to depend on the balance between the cost of remaining in one habitat and that of moving to another (Bonte et al., 2012). The high dispersal rate observed for the endogeic species when soil was poorly engineered suggests that in this case the cost of engineering activities may be higher than the cost of dispersal. However, this balance seemed to be gradually reversed when soil was further engineered, suggesting the existence of a trade-off between activities leading to soil engineering and dispersal (Bonte et al., 2012).

The positive feedback we hypothesised between the endogenic earthworm and its activities of ecosystem engineer might indicate a process of niche construction (Lewontin, 1978; Odling-Smee, 1988; Odling-Smee et al., 2013): evolution might have selected in earthworm (1) activities that allow them to change soil characteristics in a beneficial way and (2) a shift in their habitat and feeding preferences towards the modifications they impose to soils. This should lead to ecological and evolutionary feedbacks that are likely to be very influential for the whole ecology of ecosystem engineers (life-history, behaviour) (Erwin, 2008) and for ecosystem and soil properties (Raynaud et al., 2013). Our results thus suggest that dispersal and stimuli that trigger dispersal have evolved in close relation with engineering activities: decreasing dispersal in engineered soil should increase local earthworm densities and thus increases soil engineering. Such feedback may influence the selection pressure for particular dispersal strategies, as observed here. Importantly, such feedback should play an important role for the present population dynamics of earthworms, their spatial distribution, soil characteristic and heterogeneity in soil characteristics (Barot et al., 2007; Cuddington et al., 2009).

In conclusion, it would be interesting to document feedbacks between soil engineering and dispersal for other species of soil engineers in order to assess quantitatively and qualitatively the influence of these feedbacks on soil functioning and heterogeneity. These experiments contribute to a new research area merging the fields of dispersal and the ecology of ecosystem engineers.

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Annexe 10

Caro, G., A. Abourachid, T. Decaëns, L. Buono, and J. Mathieu, Is earthworms' dispersal facilitated by the ecosystem engineering activities of conspecifics? . Biology and Fertility of Soils, 2012. 48: p. 961-965.

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SHORT COMMUNICATION

Is earthworms' dispersal facilitated by the ecosystem engineering activities of conspecifics?

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Abstract In this work, we documented the influence of earthworm's galleries on their speed of movements during dispersal events in the soil. We quantified, by using X-rays, the dispersal behaviour of earthworms in the soil. The observations were conducted in mesocosms in controlled conditions for 12 h. Our experiments revealed that during a dispersal sequence of a batch of individuals of the species *Aporrectodea terrestris* (Savigny 1826): (a) individuals used preferentially existing conspecifics' galleries, (b) individual velocity increased after each dispersal event and (c) the lag time before each dispersal event did not seem to be influenced by previous dispersers. Therefore, dispersal seems to be facilitated by conspecifics' activity, which strongly supports the hypothesis of a feedback between ecosystem engineers' activity and their dispersal speed.

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Keywords Cineradiography · X-ray imagery · Conspecific facilitation · Dispersal behaviour · Earthworms' activity · Ecosystem engineering

Introduction

Earthworms have a profound influence on soil's physical and chemical properties (Zhang and Schrader 1993; Blanchart et al. 1999). Consequently, they play a central role in soil functioning and in plant growth (Lee 1985; Edwards and Bohlen 1996; Scheu 2003). Their impacts on soil functioning and soil biota through the engineering of their physical environment have been the subject of a large number of studies. In contrast, there is little information available on the consequences of the potential feedback of these activities on their own life condition, although this is expected to play an important role in earthworm ecology and activity (Odling-Smee 1995; Mathieu et al. 2010). We now need to grasp these feedbacks in order to understand the driving factors of earthworm activity and spatial distribution. Here, we propose to explore the potential feedback between the construction of galleries and the dispersal speed of earthworms in the soil.

Dispersal is a central ecological process that allows both the colonization of new habitats and the exploitation of spatially and temporally variable resources (Ronce 2007). Active dispersal of animals (opposed to passive dispersal, where individuals are transported by an external agent) involves three successive behavioural stages: departure from a breeding site, crossing to a new place and settlement (Clobert et al. 2001, 2009). A recurrent finding of evolutionary models is that dispersal rate depends on the balance between the costs and benefits of dispersal (Bowler and Benton 2005), which are strongly determined by environmental factors (e.g. habitat

quality, habitat fragmentation, patch size, density and predation) and individual life traits [e.g. age and hormonal levels (Bonte et al. 2006; Schtickzelle et al. 2006)]. In consequence, strategies that reduce these costs, such as the capacity to use cues based on conspecifics and/or environmental conditions, were selected over evolutionary times in many groups (Clobert et al. 2009). Such use of cues is not known in earthworms, but previous studies suggest it might exist (Mathieu et al. 2010; Zirbes et al. 2010, 2012). It was noticed that the products of engineering activities, such as burrows, might be used as cues by earthworms to evaluate the state of the environment.

Based on these results, the existence of a feedback between earthworm activities and their dispersal behaviour was questioned. For instance, Mathieu et al. (2010) showed that earthworm dispersal rate, during the departure stage, could be reduced when individuals were inoculated in a soil that was pre-used by conspecific individuals—which were no longer present—showing that earthworms can detect the former activity of conspecifics. Other studies showed that anecic earthworms use the galleries of conspecific individuals or of other species, but not specifically for dispersal (Capowiez 2000; Bastardie et al. 2003). These observations raise the question of the influence of earthworm activities on the speed of their movement during the second stage of dispersal (crossing stage).

In this work, we specifically investigated the potential feedback between earthworms' activities and their dispersal rate by addressing three questions: (a) Do individuals use preferentially pre-existing conspecific galleries to disperse? (b) Does dispersal velocity increase in a soil where conspecific has already dispersed? and (c) Is dispersal triggered by the departure of previous dispersers (like in a collective movement)? To answer these questions, we developed a new technique based on X-ray imagery that allows to take pictures of earthworms in the soil and to quantify their behaviour.

Materials and methods

We used the species *Aporrectodea terrestris* (Savigny 1826), more commonly called *Aporrectodea giardi* (Ribaucourt 1901), an anecic species (size, 130–170 mm; mean weight, 3.3±0.9 g), which lives in the soil and feeds on surface litter (Bouché 1972, 1977). Adult earthworms were collected in the north of France (49°27′ N, 1°4′ E) and were kept in suitable soil (see below) at low density (1.5 individuals per litre of soil), at 15°C during the day and 10°C at night. All earthworms were used once and were adults during the experiments. To overcome the problem of transparency of worms to X-rays and to have an accurate tracking point, we tagged individuals subcutaneously with a rod of lead of 2 mm. Tags do not affect the growth of earthworms (Butt and Lowe 2007). Preliminary tests comparing dispersal behaviour between tagged and control individuals (not tagged) showed no effect on dispersal response (unpublished data).

Two types of soil were used for the experiments: an unsuitable and a suitable soil. The unsuitable soil consisted of a very sandy soil with low pH (Table 1) collected in an area deprived of earthworms in the forest of Fontainebleau, France (48°24′ N, 2°44′ E). The suitable soil (Table 1) contained more organic matter and clay than the unsuitable soil and was sampled in a grassland of the IRD research centre of Bondy, France (48°54′ E, 2°29′ N). Both soils were air dried, sieved at 2 mm and rewetted manually to 25 % of humidity (on a mass basis). The preference of earthworms for the suitable compared to the unsuitable soil was tested in a previous experiment (Mathieu et al. 2010).

The experiments were conducted in mesocosms following the procedure developed in Mathieu et al. (2010). Mesocosms consisted of dispersal corridor of 100 cm long, 20 cm wide and 20 cm height. They were composed of three equal parts (Fig. 1): (1) the inoculation section, which was filled with unsuitable soil; (2) the intermediate 'crossing section', composed of unsuitable soil and (3) the arrival section composed of suitable soil. All soils were disposed at density of 1±0.1 $g cm^{-3}$. This setup triggered dispersal as individuals tend to disperse from habitats of low quality (Mathieu et al. 2010). It allows reproducing the three stages of dispersal: departure, crossing and settlement in a suitable site (Clobert et al. 2009). Earthworms were filmed in the crossing section, which was thinned by 40 % to allow earthworm detection by X-rays. Each experimental unit was closed over by a tarp, to keep humidity and to prevent the worms from escaping. The experiment was replicated five times with different experimental units and different individuals each day.

In each replicate, 10 individuals were inoculated at the same time in the inoculation section (Fig. 1). In order to prevent any contact between individuals during the inoculation, we ensured that each individual was inoculated at a sufficient distance from the others (10 different locations with a distance of at least 5 cm from each other), and we checked that all individuals entered into the ground (on average, 5 min).

Table 1 Selected properties of the used soils in the experiments

Soil properties (unit)	Unsuitable soil	Suitable soil
Clay (%)	4.7	15.7
Silt (%)	18.5	13.4
Sand (%)	76.8	70.9
Organic C ($g kg^{-1}$)	8.5	28.1
Total N ($g kg^{-1}$)	0.33	2.61
C/N	25.8	10.8
Organic matter ($g kg^{-1}$)	14.6	48.6
pH	3.8	7.5
CEC (Metson) ($cmol kg^{-1}$)	2.9	11.7

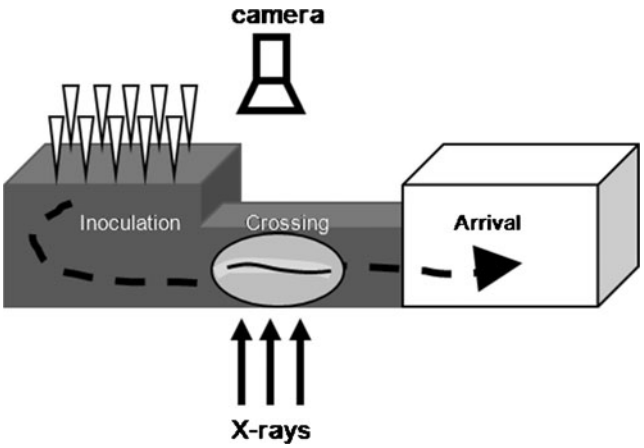

Fig. 1 Experimental design of the study (100 cm long; 20 cm wide; 20 cm height, 8 cm height for the thinned section); *Grey area* = unsuitable soil; *white area* = suitable soil. The clearest *circle* represents the observed area. Each *triangle* represents an inoculation point, where a single earthworm was introduced. *Arrows* represent the section observed by X-rays, which have been captured by a camera. A *dashed arrow* symbolizes the direction of movement

The X-ray filming device was composed of a videofluoroscopy machine (Philips Diagnostic C generator with a Basler A 504 K with digital video camera recorder), which could not be moved. The X-rays were generated at 1.6 mA and 54.0 kV, which allowed seeing the worms in the soil within a 20-cm radius. After inoculation of the 10 earthworms, snapshots of the first 20 cm (in length) of the crossing section were taken (Fig. 1) every minute for 12 h. Dispersal events occurred without any artificial stimulation, which could have disturbed the dispersal behaviour of earthworms. In consequence, we observed at most three passages in each replicate.

The X-ray filming device allowed us to take pictures of earthworms and their galleries in the soil and to measure their dispersal velocity as well as the lag time between subsequent passages of individuals within replicates. For each individual, we determined the entrance and exit time in the observed section (approximately 315 cm²), which was centred on the crossing section. The velocity (V) was determined by the ratio between the travelled distance and the time required to travel over the corresponding distance. The difference of time between the moment where a worm left the observed section and the moment the next conspecific entered was used to calculate the lag time between two crossing events. The ratio $Vn+1/Vn$ between the velocity during the passage $n+1$ and during the passage n was used to quantify the relative change in the dispersal velocity. We evaluated the link between the different components of dispersal behaviour (movement speed and lag time between dispersal events) and the order of passage or to the presence/absence of gallery with a linear regression model. All analyses and graphs were performed with the software R (Ihaka and Gentleman 1996).

Results and discussion

Despite the low number of dispersal events, we can clearly see that after the first crossing event, a majority (84 % in the second dispersal event and 100 % in the third) of the new dispersers used a gallery already built, rather than burrowing a new one. One individual started a new gallery but ended up in an existing gallery.

Our results showed a striking increase of dispersal velocity due to previous earthworm's activities. We observed a significant increase of dispersal velocity along the sequence of dispersal events (linear regression $r^2=0.58$, p value=0.002, Fig. 2): during the gallery construction phase (see the attached accelerated video file about gallery construction), the average velocity was 0.6±0.3 cm min^{-1}, which was the lowest speed observed. This result can be related to the low colonization rate of non-inhabited or previously tilled plots usually observed in the field (Butt et al. 1995; Nuutinen et al. 1997, 2006; Grigoropoulou and Butt 2010; Eijsackers 2011). Our observations of dispersal velocity are well above the observations made in earthworm-free soils (Eijsackers 2011), reporting colonization a rate of 5–8 myear^{-1} for *Aporrectodea longa* and 1.5–4 myear^{-1} for *Lumbricus terrestris*. However, a comparison between our results in experimental device to these field observations must be done with caution due to the differences in environmental conditions (spatial and temporal heterogeneity, weather and interspecific interactions), in scale (1 m versus a few kilometers) and in the length of observation (12 h versus years). However, our results provide new insights in the understanding of dispersal mechanisms of earthworms. Indeed, our observations supply evidences of the capabilities

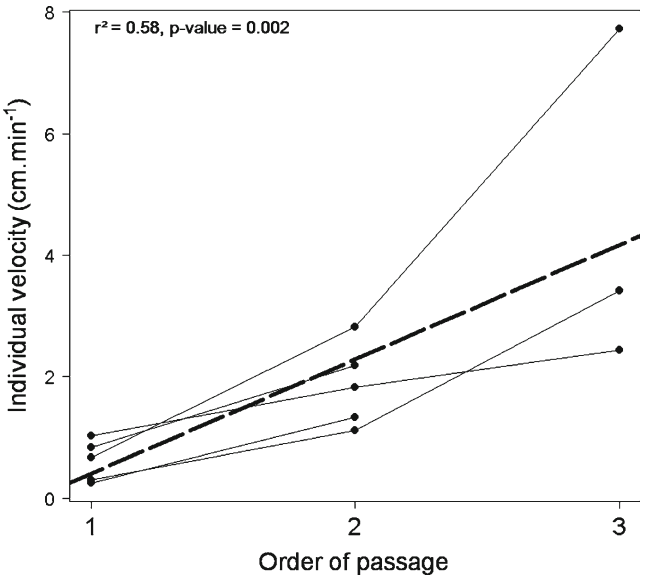

Fig. 2 Individuals' dispersal velocity (in centimetres per minute) according to their rank of passage during the dispersal sequence. A *line* links earthworms belonging to the same mesocosm. The *dashed line* represents the linear regression

of earthworms to move faster than expected from field observations.

Average velocity of second dispersal events was three times faster (1.8±0.7 cm min^{-1}) than during the first one. This result can be explained by the fact that moving in existing galleries requires much less efforts than moving in a pristine soil, as no burrowing work is required (Ehlers 1975; Edwards and Lofty 1980; Hirth et al. 1997). During the third passage, earthworms exclusively used existing galleries, and the average velocity was then even higher (4.5±2.8 cm min^{-1}), than during the first and second dispersal events. This can hardly be explained by a decrease of burrowing costs because they are low during the second and third dispersal events. This suggests that the observed increase in dispersal velocity along dispersal sequence should be triggered by another mechanism. Non-selective detection of conspecific activity, like detection of empty spaces in the soil, would result only in the preferred use of conspecifics' galleries. It cannot explain the increase in speed between the second and third dispersal events. The most parsimonious explanation for this increase in speed is the retrieving of cues related to conspecifics' activity or presence, such as chemical cue (Schmidt Jr 1955; Ressler et al. 1968; Jiang et al. 1990). These cues could be non-specific by-products of earthworms' transit in the galleries (like urea, faeces or the results of the interaction between mucus, microflora and soil on the walls of the galleries; Pan et al. 2010) or less likely could be specific molecules like pheromones, like in ants (Dorigo et al. 1996).

The lag time between two consecutive passages was apparently not influenced by the presence of galleries or the number of previous departures (p value=0.5, linear model). Therefore, it seems that dispersal was not induced by the existence of galleries or by social interactions during the departure of conspecifics, in contrast to previous observations (Zirbes et al. 2010).

Conclusion

Our results show that earthworm dispersal movements are much faster in areas previously engineered (i.e. burrowed) by conspecifics. Individuals selectively follow existing galleries rather than building new galleries, raising the question of the mechanisms involved in the localization of the galleries. This shows that earthworms' dispersal in soil is facilitated by their own activity, highlighting the existence of a feedback between engineering activities and dispersal velocity. It would be interesting to determine if this feedback is accidental (not specific, like autogenic engineers; Jones et al. 1994; Jouquet et al. 2006) or intended. Finally, our findings ask the question about the potential role of dispersal facilitation in community dynamic of earthworms and the influences of this facilitation between different earthworm species or ecological categories, especially regarding colonization pattern of new habitats (Uvarov 2009; Eijsackers 2011).

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  - Caroline Thiollet (UPMC – AgroparisTech) - Mécanismes de communication chez les vers de terre
  - Raphael Marichal (UPMC – ENS) - Contraintes stoechiométriques chez les vers de terre
  - Christophe Lecarpentier (UPMC) - Mécanismes de communication chez les vers de terre
  - Gael Caro (UPMC) - Stratégies de dispersion chez les vers de terre
  - Said Damouch (UPMC) - Biogéographie des vers de terre en France
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  - 6 étudiants en M1
  - 15 étudiants en L3

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*LES * INDIQUENT LES PUBLICATIONS IMPLIQUANT LES ÉTUDIANTS QUE J'AI ENCADRÉS. LEUR NOM EST SOULIGNÉ.*

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